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Design, synthesis and characterization of poly (methacrylic acid-niclosamide) and its effect on arterial function

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ABSTRACT

We have found that niclosamide induced relaxation of constricted artery. However, niclosamide is insoluble, the low bioavailability and the resultant low plasma concentration limit its potential exertion *in vivo*. The aim of the present study is to synthesize a soluble poly (methacrylic acid-niclosamide) polymer (PMAN) and study the effects of PMAN on arterial function *in vitro* and the blood pressure and heart rate of rats *in vivo*. We synthesized the poly (methacrylic acid-niclosamide) polymer (PMAN), the chemical structure of which was identified by FTIR and ^{1}H NMR spectra. The average molecular weight and polydispersity index of PMAN were 5138 and 1.193 respectively. Compared with niclosamide, the water solubility of niclosamide in PMAN was significantly increased. PMAN showed dose-dependent vasorelaxation effect on rat mesenteric arteries with intact or denuded endothelium in phenylephrine (PE) and high K^+ (KPSS)-induced vasoconstriction models *in vitro*. The efficacy of vasorelaxant effect and the cytotoxic effect of PMAN on vascular smooth muscle cells (A10) were lower than that of niclosamide. The LD_{50} of PMAN in mice (iv) was 80 mg/kg. Venous injection of PMAN (equivalent 5 mg niclosamide per kg) showed acute reduction of the rat blood pressure and heart rate *in vivo*. In conclusion, the solubility of niclosamide was increased in the way of poly (methacrylic acid-niclosamide) polymer, which relaxes the constricted arteries *in vitro* and reduces the rat blood pressure and heart rate *in vivo*, indicating that modifying niclosamide solubility through polymerization is a feasible approach to improve its pharmacokinetic profiles for potential clinic application.

1. Introduction

Our previous studies found that mitochondrial uncouplers carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) and niclosamide ethanolamine relaxed the constricted artery [1,2], indicating that mitochondrial uncouplers could be developed as anti-hypertensive drugs potentially. CCCP is a typical mitochondrial uncoupler and only used as a pharmacological tool but not in clinic. Niclosamide is a clinically anthelmintic drug approved by FDA (USA). Niclosamide has multiple biological activities, including anthelminthic activity, antibacterial activity [3], anti-cancer activity [4–6]. Niclosamide ethanolamine, the salt form of niclosamide, prevented hepatic steatosis and insulin resistance and improved glycemic control in animal models [7]. Nevertheless, the equilibrium solubility of niclosamide in saline is only 0.249 ± 0.005 μg/mL [8]. The poor water solubility and low oral bioavailability, and the resultant low plasma concentrations of niclosamide limit its potential exertion *in vivo*. Even in ethanolamine salt form, the water solubility of niclosamide is not significantly increased yet. We found that niclosamide ethanolamine at 1 μM concentration significantly relaxed the constricted arteries *in vitro*; however, oral dose of niclosamide ethanolamine (300 mg/kg, twice daily) did not affect the rat blood pressure *in vivo* [2]. We had tried to inject niclosamide ethanolamine through vein, but the bolus injection of the solvent DMSO was a big problem.

In order to overcome the poor water solubility of niclosamide, we design a poly (methacrylic acid-niclosamide) polymer based on the chemical structure of niclosamide and the application of poly (methacrylic acid) in biomaterial fields [9–13]. We hypothesize that this polymer could increase the solubility of niclosamide and release the free niclosamide to exert its actions after being hydrolyzed *in vivo*.

Abbreviations: AIBN, 2,2-azobisisobutyronitrile; GPC, gel permeation chromatography; MAA, methacrylic acid; Nic, niclosamide; NEN, niclosamide ethanolamine; Nic-AC, 5-chloro-*N*-(2-chloro-4-nitrophenyl)-2-(2-acryloyloxy)-benzamide; PMAN, poly (methacrylic acid-niclosamide); PMAA, polymethacrylic acid; TEM, transmission electron microscopy.

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In the current study, we found that the poly (methacrylic acid-niclosamide) polymer increased the solubility of niclosamide, relaxed the constricted arteries *in vitro* and reduced the rat blood pressure after venous injection *in vivo*.

2. Experimental methods

2.1. Animals and agents

The adult Sprague-Dawley rats (male, body weight 320–350 g) and Kunming mice were purchased from Charles River (Charles River Laboratory Animal, Beijing, China). All the experimental procedures about animals were approved by the Institutional Animal Care and Use Committee of Harbin Medical University, PR China. Arterial smooth muscle cells (A10) were purchased from ATCC. Phenylephrine (PE) was purchased from Shanghai Harvest Pharmaceutical Co.LTD., China. Tetramethylrhodamine methyl ester (TMRM) and hoechst were purchased from life technology (invitrogen, Oregon, USA). Niclosamide was purchased from Jianglai Reagent Company (Shanghai, China). Methacrylic acid (MAA) and anhydrous tetrahydrofuran (THF) were purchased from aladdin[@] industrial corporation (Shanghai, China). Acryloyl chloride (AC) was purchased from energy chemical (Shanghai, China). All other chemicals were purchased from commercial suppliers.

2.2. Synthesis procedures

The synthetic route of poly (methacrylic acid-niclosamide) (PMAN) was shown in Fig. 1.

2.2.1. Synthesis of 5-chloro-N-(2-chloro-4-nitrophenyl)-2-(2-acryloyloxy)-benzamide (Nic-AC)

Niclosamide (0.01 mol) was dissolved in anhydrous THF (200 ml) and anhydrous potassium carbonate (0.025 mol) was added, then acryloyl chloride (0.018 mol) in anhydrous THF (10 ml) was added dropwisely. The solution was mixed 0.5 h below 10 °C in dark. The reaction mixture reacted at 55 °C for 16 h in dark. The insoluble substance was filtered and washed with water for four times and dried at 50 °C under vacuum.

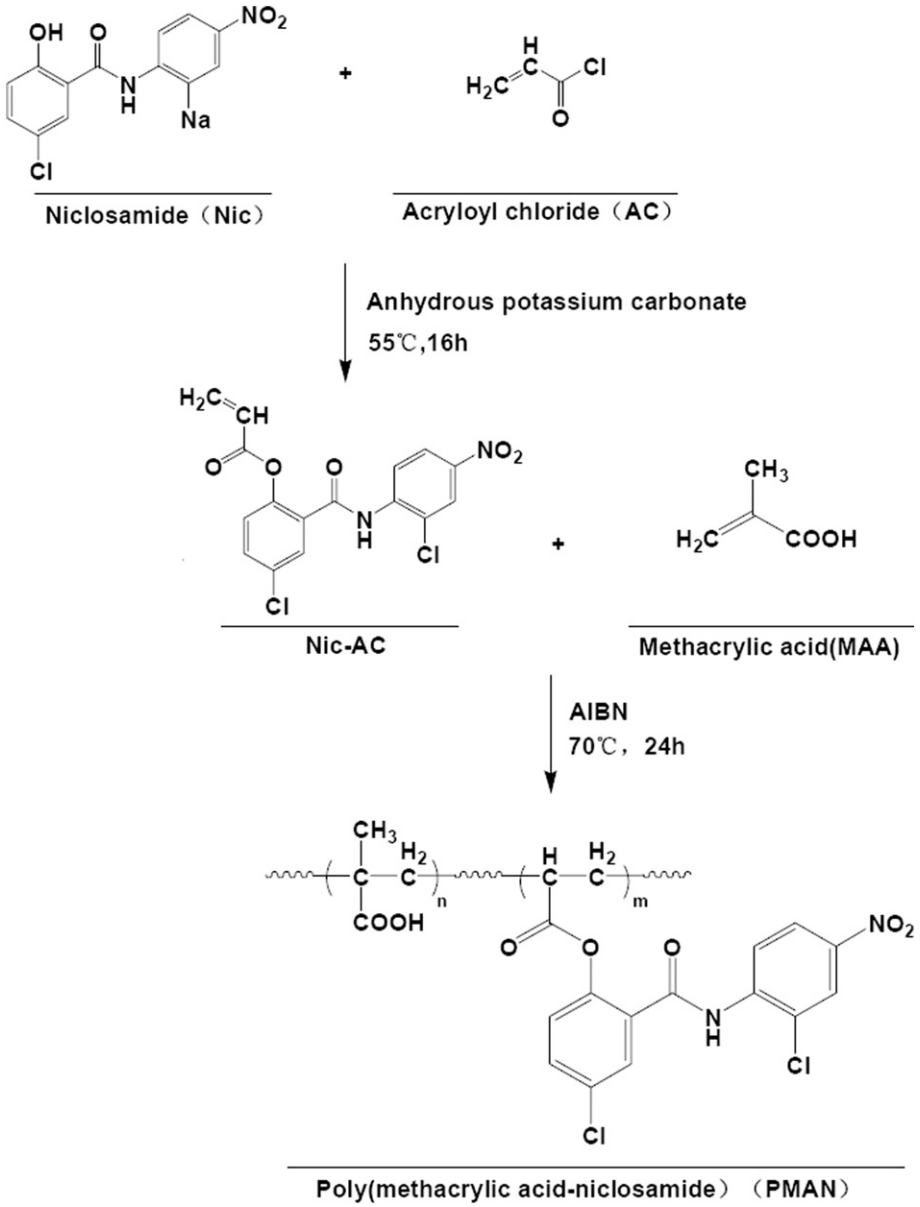

Fig. 1. Synthesis routes of poly(methacrylic acid-niclosamide) (PMAN).