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Research Paper

Octenylsuccinate hydroxypropyl phytoglycogen enhances the solubility and in-vitro antitumor efficacy of niclosamide

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ABSTRACT

Niclosamide is a promising antitumor agent, but its low aqueous solubility places limit on its antitumor efficacy. The aim of this study was to improve the solubility and dissolution of niclosamide through using octenylsuccinate hydroxypropyl phytoglycogen (OHPP), an amphiphilic dendrimer-like biopolymer. The niclosamide-OHPP solid dispersion (niclo-OHPP SD) was prepared and characterized in terms of crystallinity, molecular interactions, solubility and dissolution profile, in-vitro antitumor efficacy, and in-vitro transdermal amount. X-ray powder diffraction analysis showed the amorphous state of niclosamide in niclo-OHPP SD. FTIR showed the formation of hydrogen bonding between niclosamide and OHPP. Solubility of niclosamide with niclo-OHPP SD was about 11,914 times that of niclosamide alone, and 61% of niclosamide with niclo-OHPP SD dissolved in 3 h upon dissolution. Against three cancer cell lines, cytotoxicity assays indicated greater inhibition by using niclo-OHPP SD than by using DMSO-assisted niclosamide solution. The cumulative transdermal amount of niclosamide with niclo-OHPP SD was 5.3 times that with niclosamide alone. This study showed that the use of OHPP could provide strong support for the development of niclosamide-based drug formulations.

1. Introduction

Niclosamide (2,5-dichloro-4-nitrosalicylanilide) is an anthelmintic drug used to treat tapeworm infestations. Interestingly, recent studies have shown that niclosamide is a promising anticancer agent against various human cancers, such as leukemia, ovarian carcinoma, and breast carcinoma (Li et al., 2014). The low aqueous solubility of niclosamide; however, places an obstacle to its bioavailability as an anticancer medication, which calls for an enabling formulation to facilitate niclosamide dissolution so as to exert its bioactivities.

Several methods have been used to improve the solubility of niclosamide, including the reduction of its crystal particle size to nanoscale by wet media milling (Ye et al., 2015); co-crystallization with materials that are generally recognized as safe (Sanphui et al., 2012); complexation with *O*-phosphorylated calixarene or cyclodextrin (Bayrakci et al., 2012; Yang and de Villiers, 2005); formation of prodrug by introducing a hydrophilic functional group (Chen et al., 2013); and solubilization by polyamidoamine (PAMAM) (Devarakonda et al., 2005). While these methods have shown merits from various perspectives, a new bio-based, high-potency solubilizer would provide additional options in formulating niclosamide and other poorly water-soluble drugs.

In this study, octenylsuccinate hydroxypropyl phytoglycogen (OHPP) was used to enable niclosamide by increasing its solubility. OHPP is a derivative of phytoglycogen (PG), a naturally occurring dendrimer-like biopolymer extracted from various plants and in particular from *su1* mutant maize (Huang and Yao, 2011). Because of its highly branched structure, PG was able to harbor and disperse insoluble phytochemicals and thus to improve their solubility and permeation (Chen and Yao, 2016, 2017). In addition, chemical modifications of PG may lead to more functional derivatives. In earlier studies, phytoglycogen octenylsuccinate was used as an emulsifier to stabilize the emulsion and extend the stability of oil (Scheffler et al., 2010a). Nano-11, another PG derivative, was used as a high-efficacy vaccine adjuvant (Lu et al., 2015). Used in this study, OHPP was prepared through grafting PG particulate with hydroxypropyl and octenylsuccinate groups. It was found that OHPP was able to solubilize a number of poorly water-soluble compounds, including celecoxib, docetaxel,

Abbreviations: API, active pharmaceutical ingredient; D_2O, deuterium oxide; DMSO, dimethyl sulfoxide; FTIR, Fourier Transform-Infrared; NMR, nuclear magnetic resonance; HP, hydroxypropyl; HPLC, high performance liquid chromatography; HPSEC, high performance size exclusion chromatography; MALLS, multi-angle laser-light scattering; MS, molar substitution; MTT, 3-(4,5-dimethyl-thiazol-yl-2)-2,5-diphenyl tetrazolium bromide; M_W, weight-average molecular weight; MWCO, molecular weight cut-off; Niclo, niclosamide; OHPP, octenylsuccinate hydroxypropyl phytoglycogen; OS, octenylsuccinate; OSA, octenylsuccinic anhydride; PAMAM, polyamidoamine; PM, physical mixture; PG, phytoglycogen; RI, refractive-index; R_Z, Z-average root mean square radius; SD, solid dispersion; XRD, X-ray diffraction; ρ, dispersed molecular density; ζ, zeta-potential

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fenofibrate, griseofulvin, and resveratrol (Xie and Yao, 2018).

The primary goal of this study was to evaluate the effectiveness of OHPP to solubilize and enable niclosamide as an anti-cancer compound without the use of DMSO for dissolving. The in-vitro transdermal amount of niclosamide was also tested to study the permeation enhancement of OHPP-enabled niclosamide, which was necessary to explore the potential use of niclosamide to address skin cancers.

2. Material and methods

2.1. Material

Prostate cancer cell PC-3 (ATCC® CRL-1435™), cervix cancer cell HeLa (ATCC® CCL-2™), and lung cancer cell A549 (ATCC® CCL-185™) were purchased from America Type Culture Collection (Rockville, MD). Niclosamide was purchased from Sigma-Aldrich (St. Louis, MO). OHPP was prepared as described by Xie and Yao (2018). Soluplus® and HPMCAS were obtained from BASF Australia Ltd (Melbourne, Australia) and Shin-Etsu Chemical Co. Ltd. (Japan), respectively. Other chemicals were of reagent grade.

2.2. Transmission electron microscope (TEM) imaging of OHPP

OHPP particulates were imaged using TEM as described by Scheffler et al. (2010b) with modifications. Carbon-coated 400 mesh grid (FCF 400-Cu, EMS, PA) was glow discharged (PELCO easiGlowTM, Ted Pella Inc.) before use. Droplets of 0.01% (w/v) OHPP in pure ethanol was dried on the grid and stained by 2% aqueous uranyl acetate. Samples were imaged using a Tecnai G2 20 TEM (FEI company, Hillsboro OR) operated at 200 kV.

2.3. Phase solubility of niclosamide in OHPP solution

The phase solubility of niclosamide in phosphate buffer (pH 6.8, 58 mM) containing OHPP of various concentrations (ranging from 0 to 100 mg/mL) was measured according to Higuchi and Connons (1965). Briefly, 10 mg niclosamide was added to 1.0 mL OHPP solution to form a suspension that was agitated (100 rpm) at 25 °C for 24 h followed by centrifugation at 16,000 × *g* for 5 min. The concentration of niclosamide in the supernatant was determined using HPLC (Agilent 1100 series, Agilent Technologies, Wilmington, DE). The column used was XDB-C18 column (ZORBAX Eclipse, Agilent Technologies, Wilmington, DE) with mobile phase of acetonitrile-water-formic acid (60:40:0.1, v/v/v) at a flow rate of 1.0 mL/min, and the detection wavelength was 244 nm. A phase solubility curve was constructed by plotting the concentration of niclosamide in the supernatant against the concentration of OHPP.

2.4. Preparation of niclosamide-solubilizer solid dispersion

The preparation of niclosamide-solubilizer solid dispersion involved a procedure that niclosamide and the carrier (OHPP, HPMCAS, or Soluplus®) were dissolved in ethanol and spray-dried to collect the solid. Three grams of niclosamide and 9.0 g solubilizer were both dissolved in 600 mL ethanol, and the solution was spray-dried using a Büchi mini-spray dryer B-290 (BÜCHI, Switzerland) equipped with nitrogen purge for use with organic solvents. The inlet and outlet temperatures were 90 °C and 57–60 °C, respectively. The feed rate was 6 mL/min with nitrogen gas flow rate of 350 L/h. The solvent in the nitrogen gas from the outlet was condensed and collected using Büchi Inert Loop B-295.

The physical mixture of niclosamide and OHPP (niclo/OHPP PM) was prepared through blending niclosamide and OHPP solids at the weight ratio of 1:3. Both niclo-solubilizer SD and niclo/OHPP PM solids were stored at −20 °C for later experiments.

2.5. X-ray powder diffraction analysis

X-ray diffraction was used to study the crystallinity of niclosamide, OHPP, niclo/OHPP PM, and niclo-OHPP SD. The instrument was a Shimadzu-6000 XRD diffractometer (Shimadzu Corp., Japan). Diffraction patterns were collected using Ni-filtered CuKα ($\lambda = 1.5418$ A°) radiation with X-ray tube operated at 40 kV and 30 mA. All specimens were scanned in the range of $10^\circ \leq 2\theta \leq 35^\circ$ with a scanning speed of 2° (2θ)/min.

2.6. FTIR analysis

The FTIR spectra were obtained using a Thermo-Nicolet Nexus 470 FTIR spectrometer (Nicolet, Thermo, USA) equipped with a Smart OMNI-sampler. The scanning range was 800–4000 cm^{-1} with resolution of 2 cm^{-1} and 36 accumulations. FTIR spectra of niclosamide, OHPP, niclo/OHPP PM, and niclo-OHPP SD were recorded.

2.7. Solubility assay

In this study, the solubility of niclosamide was defined as its concentration in the supernatant collected through centrifuging (16,000 × *g*, 5 min) the niclosamide-containing dispersion. Individual solids of niclosamide alone or solid dispersions, each containing 2.5 mg niclosamide, were each dispersed in 1.0 mL phosphate buffer (58 mM, pH 6.8) through 2 h agitation in a shaking water bath (37 °C, 100 rpm) (SHEL LAB Models WS27, VWR International, PA). The dispersions were then subjected to centrifugation, and the concentrations of niclosamide in the supernatants were determined using HPLC. In addition, the dependence of niclosamide solubility on the amount of niclo-OHPP SD solid added to aqueous buffer was evaluated by dispersing the SD solid in phosphate buffer at concentrations of 400 μg/mL, 4.0 mg/mL, 8.0 mg/mL and 40.0 mg/mL.

2.8. Dissolution assay

The dissolution profile of niclosamide-containing solids was determined using USP Apparatus II (paddle) (Varian VK7025, Varian Inc, Cary, CA). Each sample containing 50 mg niclosamide was added to 500 mL phosphate buffer (pH 6.8, 58 mM), and the dispersion was stirred at 100 rpm. A 2-mL aliquot was withdrawn from each vessel at 5, 10, 15, 30, 60, 90, 120, and 180 min, and centrifuged at 16,000 × *g* for 5 min. The concentration of niclosamide in the supernatant of each aliquot was determined using HPLC.

2.9. Cancer cell lines inhibitory assay

In this study, niclosamide solid was first dissolved in DMSO to prepare stock solution (10 mg/mL). The stock solution was then diluted using cell culture media to form a series of concentrations from 0.476 ng/mL to 125 μg/mL. These solutions were termed as “DMSO-assisted niclosamide solutions”. For niclo-OHPP SD solutions, 10.0 mg solid was dispersed in 1.0 mL phosphate buffer (pH 6.8, 58 mM) through 10 min vortexing followed with centrifugation (16,000 × *g*, 5 min). The supernatant collected (1723.6 ± 101.4 μg/mL) was diluted to 0.328 ng/mL to 86.1 μg/mL.

Cytotoxicity of DMSO-assisted niclosamide solutions and niclo-OHPP SD solutions on PC-3 cells, HeLa cells, and A549 cells were assessed through MTT (3-(4,5-dimethyl-thiazol-yl-2)-2,5-diphenyl tetrazolium bromide) test according to Venkatesan et al. (2011). The cells were seeded in 24-well culture plates at the density of 3×10^4 cells per well of 500 μL culture medium (F-12K with 10% FBS for PC-3 and A549, DMEM with 10% FBS for HeLa). The cells were allowed to adhere and grow for 24 h at 37 °C in an incubator supplied with 5% CO_2/95% air humidified atmosphere, after which the culture medium was aspirated and replaced with 500 μL fresh medium prepared using DMSO-