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Research paper

Poly(ethylene glycol)-Radix Ophiopogonis polysaccharide conjugates: Preparation, characterization, pharmacokinetics and in vitro bioactivity

Xiao Lin [a,c], Shuo Wang [a], Yan Jiang [b], Zhuo-jun Wang [c], Gui-lan Sun [c], De-sheng Xu [b,*], Yi Feng [a,**], Lan Shen [c]

[a] Engineering Research Center of Modern Preparation Technology of TCM of Ministry of Education, Shanghai University of TCM, Shanghai, China
[b] Shuguang Hospital, Shanghai, China
[c] College of Chinese Materia Medica, Shanghai University of Traditional Chinese Medicine, Shanghai, China

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ABSTRACT

Radix Ophiopogonis polysaccharide (ROP), a natural graminan-type fructan with Mw of ~5 kDa, had been found to have an excellent anti-myocardial ischemic activity. However, its rapid renal excretion following administration remarkably limits its efficacy and clinical use, which makes necessary the development of an effective delivery system. In this article, the feasibility of PEGylation to solve this problem was examined. A moderate coupling reaction between the hydroxyl-activated ROP and the amino-terminated mPEG was chosen to PEGylate ROP. Five different mPEG-ROP conjugates (with mPEG of molecular mass 2, 5 or 20 kDa) were prepared, purified, characterized and evaluated in pharmacokinetics and in vitro bioactivity. Results showed that only when the apparent molecular weight of the conjugate approached to a certain value, would its plasma elimination reduce abruptly. In general, the conjugation caused the reduction in the bioactivity of ROP; however, well-preserved bioactivity was observed when the grafting degree of the conjugate was lower. Among the five conjugates studied, the one with an average 1.3 mPEG (20 kDa) residues per single ROP was found to be satisfactory both in plasma retention and in bioactivity. It had a 47.4-fold increased elimination half-life and preserved approximately 74% of the bioactivity of ROP; moreover, the decrease in bioactivity is not significant. These findings demonstrate that PEGylation would be a promising approach for improving the clinical efficacy of ROP by prolonged retention in plasma.

1. Introduction

Polysaccharides have been generating considerable interests due to their plentiful bioactivity and low toxicity. To date, hundreds of polysaccharides from a variety of sources such as animals, plant cell walls and fungal cells have been discovered. Carbohydrates are an essential part of every cell surface and are crucial in cell surface recognition and information transfer, which makes researches on carbohydrate-based drugs become an increasingly active area in drug discovery, especially with a rather recent development in our understanding of fundamental glycobiology [1]. Radix Ophiopogonis polysaccharide (ROP), a natural graminan-type fructan with a weight average molecular weight of 4800 Da, a polydispersity index of 1.41, a backbone composed of Fruf (2 → 1) and a branch of Fruf (2 → 6) Fruf (2 → per average 2.8 of main chain residues [2] (Fig. 1a), had been found to have the anti-myocardial ischemic activity by a variety of animal and cultured cell models [3–5]. By in vivo animal models, it was found that ROP could protect against myocardial ischemic damage caused by isoprenaline in rats [3,4] and prevent myocardiocyte death induced by coronary artery ligation in rats [5]. By in vitro animal model, it was found that ROP could alleviate ischemia/reperfusion injury in isolated guinea pig myocardia [4]. By cultured cell models, it was found that ROP could protect myocardial cells and human microvascular endothelial cells (HMEC-1) from damage induced by oxygen and glucose deprivation, promote the tube formation and migration of HMEC-1, up-regulate the mRNAs and proteins of sphingosine kinase 1 and S1P receptor 1 and induce the expression of basic fibroblast growth factor and the phosphorylation of Akt and ERK in HMEC-1 [5].

Abbreviations: ROP, Radix Ophiopogonis polysaccharide; mPEG, poly(ethylene glycol) monomethyl ether; FITC, Fluorescein isothiocyanate; ${}_iP_{jk}$-R, mPEG-grafted ROP with an average *i* mPEG (*j* kDa) residues per single ROP; DMAP, 4-*N*,*N*-(dimethylamino) pyridine; DMSO, dimethyl sulfoxide; FBS, fetal bovine serum; bFGF, basic fibroblast growth factor; HMEC-1, human microvascular endothelial cells; HPGPC, high-performance gel permeation chromatography; OGD, oxygen and glucose deprivation.

* Corresponding author. Shuguang Hospital, 185 Pu An Road, Shanghai 200021, China. Tel./fax: +86 21 53825761.
** Corresponding author. Engineering Research Center of Modern Preparation Technology of TCM of Ministry of Education, Shanghai University of TCM, Shanghai 201203, China. Tel./fax: +86 21 50796201.
E-mail addresses: shutcm@163.com (D.-s. Xu), yy090503@163.com (Y. Feng).

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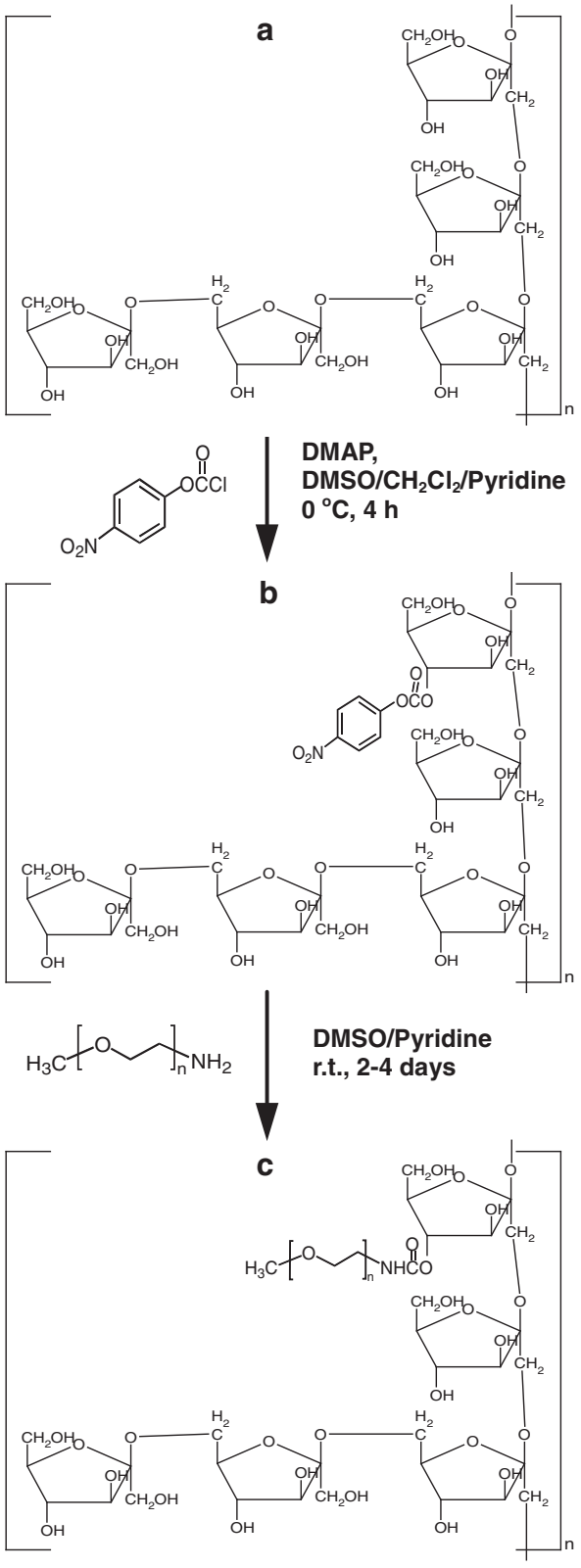

Fig. 1. Synthetic route for PEGylation of ROP. (a) ROP, (b) hydroxyl-activated ROP and (c) PEGylated ROP.

However, due to its unsuitable hydrodynamic size (~2 nm), which is larger than the intercellular space of intestinal epithelium (~1.5 nm) and much smaller than the sieving threshold of the glomerular capillary wall (~10 nm) [6], as well as its high hydrophilicity and low protein binding, ROP was poorly absorbed after oral administration, while following i.v. administration, it was very rapidly excreted by kidney [7,8]. These undesirable pharmacokinetic properties limit to a large extent its efficacy and clinical use. Many carbohydrate-based drugs such as phosphosulfomannan and its analogues [9], heparin and its mimetics [10,11] and dermatan sulfate [12] also encounter such problems, necessitating the development of an effective delivery system to improve their pharmacological action and also to reduce the administration frequency for patients.

PEGylation, the process of covalent attachment of polyethylene glycol (PEG) polymer chains to a therapeutic molecule, is now established as the method of choice for improving the pharmacokinetics and pharmacodynamics of parenteral agents. It has been successfully applied to small molecule drugs, oligonucleotides, polypeptides, proteins and long-circulating colloidal drug delivery systems [13,14]; however, although there are some reports on the application of PEGylated polysaccharides as drug carrier [15,16] or surfactant [17] and recently on the technology of glycoPEGylation for the site-directed PEGylation of polypeptides and protein [18,19], there are few if any reports on the application of PEGylation in carbohydrate-based drugs. In this study, five different PEG-ROP conjugates (with PEG of molecular mass 2, 5 or 20 kDa) were prepared, purified, characterized and evaluated in pharmacokinetics and in vitro bioactivity. The feasibility of PEGylation of carbohydrate-based drugs was tested on ROP for the first time.

2. Materials and methods

2.1. Materials, cells and animals

ROP was prepared by extraction from the tube root of *Ophiopogon japonicus* (Cixi, Zhejiang province, China) with water, followed by ethanol precipitation, chromatographic purification using DEAE Sepharose Fast Flow and Sephadex G-25 columns (Pharmacia, Uppsala, Sweden) in tandem and finally lyophilization. ROP was dried in a vacuum oven at 60 °C for 8 h before use. Linear amino-terminated poly(ethylene glycol) methyl ethers (mPEG-NH_2) were purchased from Jenkem technology Co., Ltd. (Beijing, China). *p*-nitrophenyl chloroformate and 4-*N*,*N*-(dimethylamino) pyridine (DMAP) were purchased from Fluka (Buchs, Germany). Fluorescein isothiocyanate (FITC) was purchased from Sigma (St. Louis, MO, USA). Extra dry dimethyl sulfoxide (DMSO) was purchased from Acros Organics (Geel, Belgium). Dichloromethane (CH_2Cl_2) and pyridine from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) were dried over CaH_2 and KOH, respectively, and distilled prior to use. The cell culture medium MCDB131, fetal bovine serum (FBS) and all other cell culture supplements were purchased from GIBCO-BRL (Grand Island, NY, USA). Basic fibroblast growth factor (bFGF) was obtained from Biosource (Owensboro, KY, USA). Matrigel was purchased from BD Biosciences (Bedford, MA, USA). All other chemicals were of reagent grade and purchased from commercial sources.

Human microvascular endothelial cells (HMEC-1) were obtained from Institute of Biochemistry and Cell Biology, Chinese Academy of Sciences, Shanghai, China. Male Sprague–Dawley rats, approximately 8–9 weeks old at dosing (300–360 g), were supplied by Lab Animal Center of Shanghai University of Traditional Chinese Medicine, Shanghai, China. They were kept in an environmentally controlled breeding room for 6–7 days before starting the experiments and fed with standard laboratory food and water ad libitum. The Animal Ethical Experimentation Committee of Shanghai University of TCM, according to the requirements of the National Act