Carbohydrate Polymers 143 (2016) 139–148

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Anti-HBV activity and mechanism of marine-derived polyguluronate sulfate (PGS) *in vitro*

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ARTICLE INFO

Article history:
Received 19 October 2015
Received in revised form 25 January 2016
Accepted 28 January 2016
Available online 4 February 2016

Keywords:
Polyguluronate sulfate
Hepatitis B virus
Anti-viral activity
Interferon
Raf/MEK/ERK pathway

ABSTRACT

Polyguluronate sulfate (PGS) is a low molecular-weight sulfated derivative, which has a structure of 2,3-O-disulfated-1,4-poly-L-guluronic acid (PG) with about 1.5 sulfate per sugar residue. Herein, our results showed that PGS effectively inhibited the expression and secretion of HBsAg and HBeAg in HepG2.2.15 cells. PGS could bind and enter into HepG2.2.15 cells to interfere with HBV transcription rather than blocking HBV DNA replication. Moreover, PGS also enhanced the production and secretion of interferon beta (IFN-β) in HepG2.2.15 cells. Cellular NF-κB and Raf/MEK/ERK signaling pathways were also involved in the anti-HBV actions of PGS. Thus, PGS may inhibit HBV replication through upregulating the NF-κB and Raf/MEK/ERK pathways to enhance the interferon system. In summary, PGS merits further investigation as a novel anti-HBV agent aimed at modulating the host innate immune system in the future.

1. Introduction

Hepatitis B is a common and serious chronic disease caused by hepatitis B virus (HBV), which is an enveloped DNA virus that can impair the host immune response and results in viral persistence and disease chronicity (Ratnam & Visvanathan, 2008; Renate et al., 2004). More than 350 million persons are suffering from HBV worldwide, and over one million people die each year from chronic hepatitis, cirrhosis, and hepatocellular carcinoma caused by HBV infections (Lau & Bleibel, 2008; Lavanchy, 2004). In present, the commonly used therapeutic drugs for chronically infected hepatitis B patients are interferons and nucleoside analogs, such as interferon α and Lamivudine. However, the clinical efficacy of these drugs was often constrained by their side-effects and drug resistances (Kim et al., 2008; Merle & Trepo, 2001; Sede et al., 2012). Therefore, the development of novel anti-HBV drugs with high efficiency and low toxicity is urgently needed.

Alginate, a soluble acidic polysaccharide extracted from brown seaweeds, is composed of a central backbone of poly-D mannuronic acid (PM), poly-L-guluronic acid (PG) and alternate residues of D-mannuronic acid and L-guluronic acid (Mabeau & Kloareg, 1986). Polyguluronate sulfate (PGS) is a low molecular-weight sulfated polysaccharide prepared by chemical sulfation of PG (Zhao et al., 2007). Alginate polysaccharides have recently been demonstrated to possess broad inhibitory activities against viruses, such as HSV (Mandal et al., 2008) and HIV (Thuy et al., 2015). Marine sulfated polysaccahride 911, derived from alginate, was reported to be able to improve the humoral and cellular immunity in mice, and inhibit the level of HBV DNA in HepG2.2.15 cells (Jiang, Xu, & Li, 2003). Although it is known that alginate derivatives can promote the immune response and inhibit the replication of HBV (Berven et al., 2013; Jiang et al., 2003), there have been few reports on the anti-HBV activities of PGS, the sulfated derivative of alginate.

The aim of this study was to investigate the inhibitory effects and mechanisms of PGS against HBV. We firstly evaluated the effects of PGS on the production of HBV antigens, intracellular HBV mRNA and DNA levels using HepG2.2.15 cells. Then the influence of PGS on the interferon system and its associated signaling pathways was evaluated. PGS was found to be able to effectively inhibit the expression and secretion of HBsAg and HBeAg in HepG2.2.15 cells. The anti-HBV activity of PGS might be associated with appropriate activation of NF-κB and Raf/MEK/ERK signaling pathways.

Abbreviations: 3TC, lamivudine; CC_{50}, concentration of 50% cytotoxicity; DP, degree of polymerization; ELISA, enzyme-linked immunosorbent assay; FCM, flow cytometry; HBeAg, HBV e antigen; HBsAg, HBV surface antigen; HBV, hepatitis B virus; HNF4α, hepatocyte nuclear factor 4α; IFN-β, interferon beta; MAPK, mitogen-activated protein kinases; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; NF-κB, nuclear factor-kappa B; PGS, Polyguluronate sulfate.

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http://dx.doi.org/10.1016/j.carbpol.2016.01.065
0144-8617/

2. Materials and methods

2.1. Reagents and cells

Polyguluronate sulfate (PGS) and FITC-labeled PGS (PGS-FITC) were prepared in our lab (Zhao et al., 2007). Lamivudine (3TC) was purchased from Sigma (St Louis, MO, USA). HBsAg and HBeAg enzyme immunoassay (ELISA) kits were purchased from Kehua Bio-engineering (Shanghai, China). Human interferon β (IFN-β) ELISA Kit was purchased from Wuhan Colorful Gene Biological Technology (Wuhan, China). The primary antibodies against phosphorylated NF-κB, MEK, ERK1/2, and p38 proteins, and β-actin antibody were purchased from Cell Signaling Technology (Danvers, MA, USA). The inhibitors of NF-κB (BAY 117082), MEK (U1026) and p38 (SB 203580) were obtained from Beyotime (Nantong, China). HepG2.2.15 cells were maintained in DMEM medium supplemented with 10% fetal bovine serum (FBS) and 200 μg/mL of G418, and incubated at 37 °C in an atmosphere of 5% CO_2.

Table 1
The NMR data of PG and PGS.

Compound	^{13}C NMR							
	C-1	C-2	C-2S	C-3	C-3S	C-4	C-5	C-6
PG	100.72	64.78		68.84		79.97	66.99	175.29
PGS	99.44	62.48	68.74	68.30	73.16	77.78	66.39	171.62

Compound	^{1}H NMR				
	H-1	H-2	H-3	H-4	H-5
PG	4.91	3.78	3.87	3.97	4.32
PGS	5.27	4.57	4.75	4.56	4.70

2.2. Analytical methods

The structure of PGS was verified by Fourier transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR) analysis. The FT-IR spectrum was recorded on a Nicolet Nexus 470 infrared spectrometer in the region between 400 and 4000 cm^{-1}

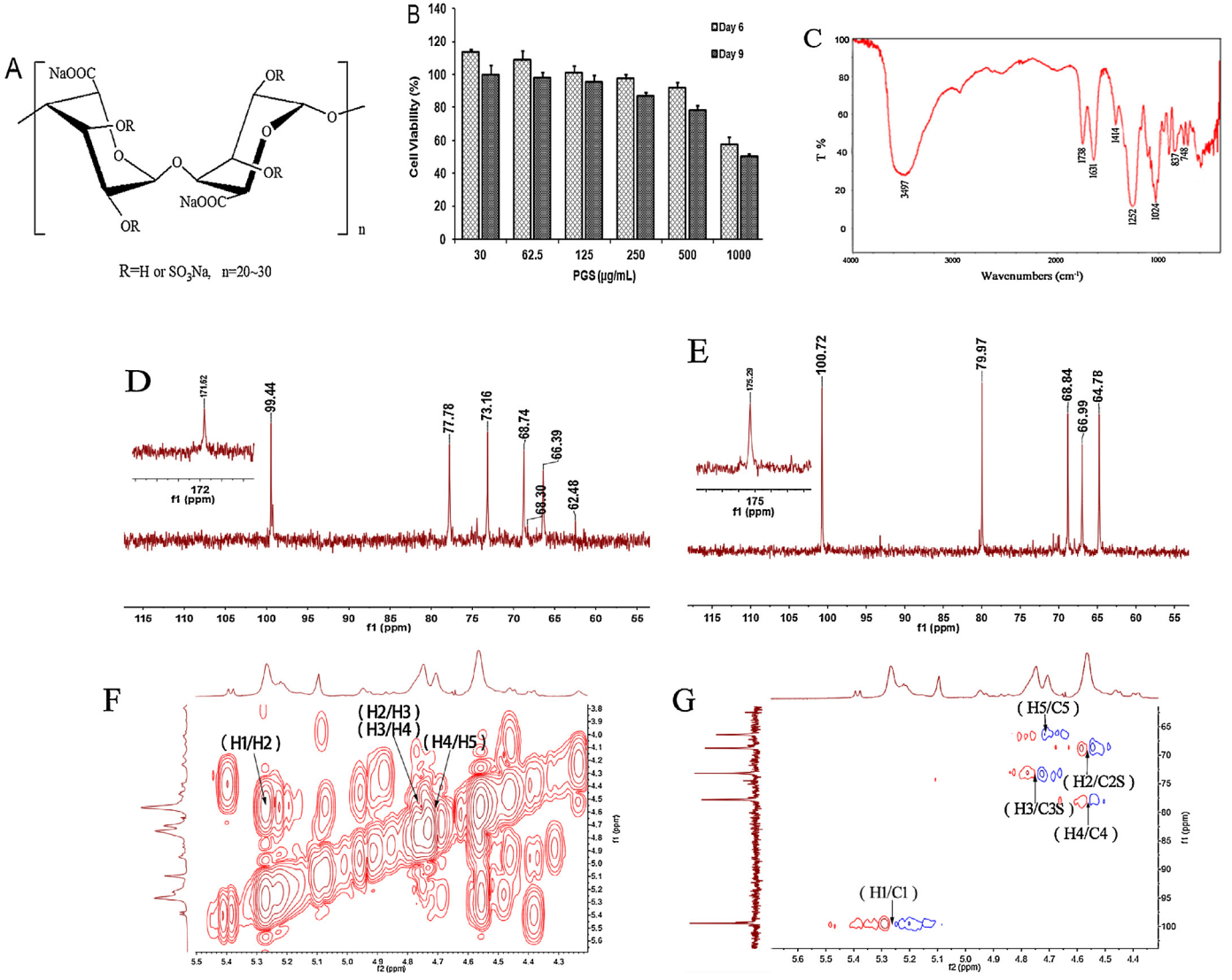

Fig. 1. Chemical structure and cytotoxicity assay of PGS. (A) Schematic illustration of PGS structure. The degree of polymerization (DP) of PGS is about 20–30. R=H, SO_3Na. (B) Cytotoxicity of PGS in HepG2.2.15 cells. HepG2.2.15 cells were cultured in the presence of PGS at indicated concentrations for 6 and 9 days. The cell viability was measured by MTT method. Values are means ± S.D. (n = 4). (C) The IR spectrum of PGS. The FT-IR spectrum was recorded in the region between 400 and 4000 cm^{-1} with a KBr pellet under dry air at room temperature (RT). (D, E) The ^{13}C NMR spectra of PGS (D) and PG (E). The ^{13}C NMR analysis was recorded at RT for samples (60 mg/mL) dissolved in D_2O in a Agilent DD2 superconducting NMR spectrometer, operating at 125 MHz for ^{13}C. (F, G) The two-dimensional NMR spectra of PGS. The spectra of NMR analysis were recorded at RT for samples (60 mg/mL) dissolved in D_2O in a Agilent DD2 superconducting NMR spectrometer, operating at 500 MHz for COSY (F) and HSQC (G).