Preparation and activity of glycosylated acetylsalicylic acid

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ABSTRACT

The glycosylated acetylsalicylic acid was prepared with bromo-β-D-galactose and acetylsalicylic acid. It indicated that the glycosylated acetylsalicylic acid had lower cytotoxicity than underivatized acetylsalicylic acid, and might selectively display anticancer activity in this situation that had enzyme or no enzyme.

1. Introduction

Acetylsalicylic acid, also known as aspirin, is a antipyretic analgesic with a long history. We all know that this medicine can be used to treat colds, fever, headaches, toothache, joint pain, and rheumatism. It also can inhibit platelet aggregation, prevent and treat ischemic heart disease, cardiopulmonary infarction, cerebral thrombosis and other diseases (De and Renda, 2012). In addition, it has shown that acetylsalicylic acid has anti-cancer effect (Rothwell et al., 2012). The US working group for preventive services has issued a guide that acetylsalicylic acid can be used as the primary prevention of cardiovascular disease and colorectal cancer. The guideline proposed for the first time that high-risk groups of non-colorectal cancer could use acetylsalicylic acid for primary prevention of colorectal cancer (Mora and Manson, 2016). It indicated that taking a certain dose of acetylsalicylic acid daily might effectively block the growth of breast cancer (Mc Menamin et al., 2017). Moreover, acetylsalicylic acid may have a certain inhibitory effect on colon cancer (Voora et al., 2016), gastrointestinal cancer (Jankowska et al., 2010), prostate cancer (Choe et al., 2012) and other cancers.

Glycosylation modifications are widely found in natural products, such as clinical antibiotic erythromycin, antiparasitic insecticide avermectins, and anticancer drug doxorubicin, which are with glycosylation modification (Huang and Mei, 2014). Glycosylation can improve the water solubility of drug, reduce toxicity and improve the activity. Moreover, it usually directly involves in the interaction of drug with the target. The activity of deglycosylated drug will be greatly affected. So, the glycosylation modification of drug plays an important role in biological activity (Huang et al., 2016). In addition, because anticancer drugs lack selectivity, tumor chemotherapy has serious side-effects. An ideal solution to the problem is the antibody-directed enzyme prodrug therapy (ADEPT) and the prodrug monotherapy (PMT) (Tietze and Schmuck, 2011). Using the principle of McAb orientation, the specific activating enzyme for prodrug is crosslinked and selectively bound to the tumor site. The prodrug can be specifically transformed into active molecule in tumor tissues, which effectively solves the problems of low concentration in tumor tissues and damage to normal tissues for drug. This prodrug design is called ADEPT. PMT method is to make anticancer drug into a prodrug containing enzyme substrate structure, and release the drug under the special enzyme action of cancer cells to play a therapeutic role. So far, there has been no report on the modification of acetylsalicylic acid by glycosylation. Therefore, we try to carry out research work in this area. β-D-galactose can be used as a vector for prodrug design (Melisi et al., 2011). So, this galactosylated acetylsalicylic acid prodrug was prepared in four-step reaction. The inhibitory activity of acetylsalicylic acid and its galactosylated prodrug was tested. In addition, their anticancer activity to cancer cells was also assayed by ADEPT and PMT.

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2. Experimental method

2.1. Preparation of peracetylated α-galactose 2

The acetic anhydride (70 mL) α-galactose (5 g) was slowly added to the reaction bottle. Then, 70 mL of anhydrous pyridine was added under stirring. This reaction was lasted for 2 h at 25 °C. After the reaction was complete, the reaction mixture was poured into ice water, which was fully stirred until room temperature. After filtration, the crude product was recrystallized with water/methanol (v/v = 1/2). Peracetylated α-galactose 2 Yield: 92%; 1H NMR (300 MHz, CDCl3): δ 6.34 (d, 1H, J1,2 1.5 Hz, H-1a), 5.63 (d, 1H, J1,2 1.5 Hz, H-1b), 5.50 (dd, 1H, J3,4 x 10 Hz, J4,5 1.3 Hz, H-4), 5.34–5.29 (m, 2H, H-2, H-3), 4.31 (dt, 1H, J5,6 6.5 Hz, H-5), 4.16–4.01 (m, 2H, H-6a, H-6b), 2.14, 2.10, 2.00, 1.98, 1.97, 1.96 (all s, 15H, 10 × C(O)CH3); 13C NMR (75 MHz, CDCl3): δ 170.8, 170.5, 170.3, 169.3, 167.5 (5 × C=O), 102.0 (C-1β), 101.5 (C-1α), 68.8 (C-5), 68.0 (C-3 and C-4), 66.2 (C-6), 61.5 (C-6), 21.3, 21.1, 20.9, 20.8, 20.6 (5 × C(O)CH3); ESI-MS: m/z = 413.3397 [M + Na]+.

2.2. Preparation of α-α-galactopyranosyl bromide 2

The peracetylated galactose 2 (5 g, 12.8 mmol) was added into the reaction bottle. Then 50 mL of hydrogen bromide-acetic acid (33%) was added dropwise at 0 °C. After reaction for 2 h at 25 °C, 150 mL of CH2Cl2 was added. The mixture was washed with ice water, and the organic layer was dried with a desiccant (MgSO4). After vacuum distillation, peracetylated α-galactosyl bromide 3 was provided in the yield of 97%. Compound 3 was hydrolyzed with 20 mL of dilute HBr solution (1 mol/L) for 2 h at 25 °C to produce α-α-galactopyranosyl bromide 4, and the yield was 90%. α-α-Galactopyranosyl bromide 4 1H NMR (300 MHz, D2O): δ 6.79 (d, 1H, J1, 3.6 Hz, H-1), 5.42 (dd, 1H, J3,4 3.2 Hz, J4,5 1.2 Hz, H-4), 5.21 (dd, 1H, J2, 10.4 Hz, H-3), 5.00 (dd, 1H, H-2), 4.33 (m, 1H, H-5), 4.15 (dd, 1H, J6,6b 11.6 Hz, J5,5e 6.0 Hz, H-6a), 3.99 (dd, 1H, J5,6a 6.8 Hz, H-6b); 13C NMR (75 MHz, D2O): δ 102.3 (C-1), 70.4 (C-5), 66.3, 66.0 (C-3/C-4), 64.9 (C-2), 59.9 (C-6).

2.3. Preparation of galactosylated acetylsalicylic acid prodrug 5

The 30 mL of anhydrous dimethyl sulfoxide was added to reaction bottle. Then, the temperature was adjusted to 40 °C. 5 mmol of acetylsalicylic acid, and its concentration was changed from 1.25 to 20 mmol/L. After 48 h, the inhibition rate to cancer cell was calculated: the inhibition rate (A) in every hole was measured at 570 nm with the Microplate Reader. The inhibition rate to cancer cell was calculated: the inhibition rate = (1 − the average value of all A values for experimental group/the average value of all A values for control group) × 100%.

2.4. Cell culture

The cell line A549 of lung adenocarcinoma was cultured in the nutrient fluid of RPMI-1640, and then it was cultured with 5% CO2 at 37 °C in incubator.

2.5. MTT colorimetric assay

The A549 cells, which were in logarithmic growth phase, were seeded in 96 well plates for 5 × 103 cells per hole. The culture medium was replaced after one day, the experimental group (sample with different concentrations), blank withered group (not inoculated for cells) and control group (solvent only with the same amount) were designed. Every group had four holes. 20 μL of MTT was added into every hole after two days. The herceptin-galactosidase conjugate was prepared according to reference (Pritsch et al., 2009). After the cultivation was lasted for 4 h in the absence or in the presence of β-D-galactosidase galactohydrolase, they were centrifuged at 1000 r/min for 5 min, and the supernatant was removed. 100 μL of dimethyl sulfoxide was added into every hole, and they were gently shaken for 10 min. The absorbance (A) in every hole was measured at 570 nm with the Microplate Reader. The inhibition rate to cancer cell was calculated: the inhibition rate = (1 − the average value of all A values for experimental group/the average value of all A values for control group) × 100%.

3. Results and discussion

Since the peracetylated monosaccharide is often used as a substrate molecule for subsequent synthesis, it is particularly important to control the terminal group configuration of peracetylated monosaccharide in the synthesis. Generally, this configuration constraint can be achieved by two methods: dynamic control and thermodynamic control. The β-configuration products are controlled by kinetics. The catalyst type, reaction temperature, feed operation and other factors can affect the rate of acetylation. So, the peracetylated monosaccharide, which has a certain α-product under dynamic control conditions, can generally be separated by recrystallization or column chromatography. The α-configuration products are thermodynamically controlled. In practice, Lewis acid or proton acid is often used as a catalyst, the resulting peracetylated monosaccharide is subjected to repeated removal and reintroduction of the terminal oxacyt glycosyl groups, and the resulting β-sugar is converted to α-sugar. Thereby, peracetylated α-sugar with a single configuration is finally produced. Here, the configuration of peracetylated monosaccharide does not affect the subsequent reaction. So, the mixture with α/β configuration was obtained. The peracetylated galactose 2 was prepared with galactose 1 and acetic anhydride using pyridine as catalyst (Fig. 1). The reaction conditions were as follows: n(galactose)/n(acetic anhydride) = 1/2 6, M(catalyst)/M(galactose) = 13/1, time = 20 h, and temperature = 25 °C. The yield was greater than 92%, and n(peracetylated α-galactose)/n(peracetylated β-galactose) = 3.2/1.

The peracetylated galactose 2 was reacted with hydrogen bromide-acetic acid (33 wt%) under stirring, time = 2 h, and temperature = 25 °C, the peracetylated α-α-galactopyranosyl bromide 3 was produced, and the yield was 97%. The peracetylated α-bromide 3 was hydrolyzed with dilute HBr solution under stirring, time = 1 h, and temperature = 25 °C, and α-α-galactopyranosyl bromide 4 was provided in 90% yield (Fig. 1).

Acetylsalicylic acid was reacted with triethylamine to produce the corresponding salt with pH = 7. The deprotected α-α-galactopyranosyl bromide 4 was reacted with the above-mentioned acetylsalicylic acid salt in anhydrous dimethyl sulfoxide, time = 5 h, temperature = 40 °C, and the target compound galactosylated acetylsalicylic acid prodrug 5 was offered. The yield was about 80% (Fig. 1).
pattern. However, the inhibition rate to cancer cell treated with galactose-acetylsalicylic acid conjugate prodrug was ranged from 6.3 to 90.2% under the same conditions by ADEPT in the presence of \(\beta\)-D-galactoside galactohydrolase. Moreover, ADEPT was better than PMT. The related results were revealed in Table 1. Therefore, it proved that the galactosylation of acetylsalicylic acid could enhance its inhibitory activity against the proliferation of cancer cells by ADEPT and PMT.

The IC\(_{50}\) of prodrug 5 and free acetylsalicylic acid was 1256.83 and 11.30 mmol/L, respectively, in the presence of \(\beta\)-D-galactoside galactohydrolase, the cytotoxicity of prodrug 5 was restored to that of the free acetylsalicylic acid. The ratio of cytotoxicity between the prodrug and the free drug was 111 (Table 2). These value is compatible with ADEPT or PMT strategy where a relatively non-cytotoxic prodrug releases a cytotoxic compound.

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