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The cough suppressive activity of sulfated glucuronoxylan from *Fagus sylvatica* L.

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ABSTRACT

Hemicellulose polysaccharides represent a large group of natural renewable polymers, however, their application potency is still low. In our study a hardwood 4-*O*-methylglucuronoxylan was isolated by alkali peroxide extraction of *Fagus sylvatica* sawdust and modified into sulfated water soluble derivative (MGXS). Highly sulfated MGXS was characterized by HPLC, FTIR and NMR spectroscopies, and tested *in vivo* on chemically induced cough reflex and smooth muscles reactivity. Farmacological tests revealed an interesting antitussive activity of MGXS. Comparative tests with drug commonly used in a clinical practice revealed that antitussive activity of MGXS was lower than that of opioid receptor agonist codeine, the strongest antitussive drug. Furthermore, the specific reactivity of airways smooth muscle was not significantly affected by MGXS, indicating thus that the polymer is not involved in the bronchodilation process.

1. Introduction

Fagus sylvatica L. (Common or European beech) is a magnificent large tree with a broad crown and smooth silver-grey bark. It is a leaf wood from Fagaceae family, widely spread in Central and Western European regions. *F. sylvatica* is considered to be the most important forest tree species from ecological and economical point of view in Central Europe. It plays a central role in current forest transition strategies [1]. It is relatively resistant to most of tree diseases and can live at least 250 years, however, usually is harvested at 80–120 years of age. *F. sylvatica* wood has a large utilization in many industrial branches. Beech wood is largely used for furniture, flooring, staircases, boards, veneers, plywoods, production of pulp in paper industry, *etc.* It is extensively used nowadays still as a fuel wood due to its high energy content.

Generally, polysaccharides–cellulose and hemicellulose are the main carbohydrate components of woody plants. The contents of cellulose and hemicelluloses vary from 40 to 50% and 15 to 30%, respectively, according to wood type [2,3]. Hemicelluloses are usually the second most abundant polysaccharides in woody plants, however, they did not find adequate industrial application till now. The dominant polysaccharide of beech hemicelluloses are *O*-acetyl-(4-*O*-methylglucurono)-xylan (MGX). It is composed of (1 → 4)-linked β-D-xylopyranose backbone irregularly branched at O-2 by single α-D-glucuronopyranosyl residue or its 4-*O*-methyl derivative [4]. Moreover, acetyl groups are localized at O-2 or O-3 of xylose residues, however, they are cleaved by alkaline treatment. Hardwood MGX is a short-branched heteropolymer of degree of polymerization in the range of 80–200 [5].

Bark of beech has found use in traditional medicine. Decoctions from *Fagus* bark were found to have antacid, antipyretic, antiseptic, antitussive, expectorant or odontalgic effects [6,7]. A tar obtained by dry distillation of the branches, is stimulating and antiseptic agent [8]. It was used internally as a stimulating expectorant and externally as an application to various skin diseases [9]. Xylans from different plant species assigned a beneficial effect on organisms. These weakly water soluble polysaccharides showed cholesterol lowering ability, decreased the risk of atherosclerosis, colorectal cancer and diabetes. Besides, they showed immunomodulatory, anticomplement, antioxidant, anti-HIV and antitussive activities [10–15].

In the present paper, the chemical characteristics and the antitussive activity of sulfated 4-*O*-methyl-glucuronoxylan were investigated. For antitussive activity tests adult guinea pigs were used as a test system.

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2. Materials and methods

2.1. Plant material and isolation of 4-O-methylglucuronoxylan

Sawdust were prepared from the trunk of the European beech (*F. sylvatica* L., family Fagaceae) cultivated in Malé Karpaty, Slovak Republic. Sawdust were air-dried and used for isolation of 4-*O*-methylglucuronoxylan. Delignification process of air-dried *F. sylvatica* sawdust and isolation procedure were performed in one step using sodium hydroxide solution and H_2O_2 according already described method [16]. The insoluble part was filtered off and the solution was precipitated with 3 vol of ethanol. The precipitate was neutralized by acidified ethanol (80%) and after filtration washed by ethanol (80%), suspended in distilled water, dialyzed and freeze-dried to give 4-*O*-methylglucuronoxylan (MGX).

2.2. Preparation of sulfated 4-O-methylglucuronoxylan

To prepare water soluble polymer for biological tests, alkali extracted and dried 4-*O*-methylglucuroxylan (1 g) was suspended in dry DMF (300 mL) and sulfated with oleum (15 mL) in DMF (30 mL) at 24–25 °C for 24 h. Reaction mixture was spilt on ice and neutralized with sodium hydroxide solution (20%), exhaustively dialyzed (MWCO 1000) and freeze-dried to give sulfated 4-*O*-methylglucuronoxylan (MGX) in a yield of 0.7 g.

2.3. Animals

Adult conscious male TRIK strain guinea-pigs, weighing 200–350 g were supplied by the Department of Experimental Pharmacology, Slovak Academy of Science, Dobra Voda, Slovakia. They were kept in the faculty animal house with food and water *ad libitum* and with a standard air conditioning system. The animals underwent a week's quarantine before starting the experiment. After adaptation of guinea pigs the experimental conditions were selected according to response to tussigen (non-responders and hypo-responders were excluded). Experimental protocol was approved by Institutional Ethics Committee of the Jessenius Faculty of Medicine, Comenius University in Martin, Slovakia, registered in Institutional Review Board/Institutional Ethic Board Office (IRB 00005636), complied with Slovakian and European Community regulations for the use of laboratory animals and follow the criteria of experimental animal's well fare. Citric acid was obtained from Sigma-Aldrich (Germany). Codeine phosphate was purchased from Lachema (Czech Republic).

2.4. General methods

Polysaccharides were hydrolyzed with 2 M trifluoroacetic acid for 1 h at 120 °C. Solutions were concentrated under diminished pressure at a bath temperature below 45 °C. The quantitative determination of the neutral sugars was carried out in the form of their alditol acetates [17], by gas chromatography on a Trace GC Ultra coupled with ITQ 900 (Thermo Scientific, USA) equipped with a Restek RT-2330-NB column (0.32 mm × 105 m), the temperature program of 80 °C (12 min)–160 °C (8 °C/min)–250 °C (4 °C/min, 25 min at 250 °C)–265 °C (20 °C/min, 10 min at 265 °C) and the flow rate of helium was 1 mL/min.

The uronic acid content was determined with the 3-hydroxybiphenyl reagent [18]. FLASH 2000 Organic elemental analyzer (Thermo Fisher Scientific, USA) for the determination of the sulphur content was used.

Fourier-transform infrared (FT-IR) spectra were obtained on a NICOLET Magna 750 spectrometer with DTGS detector and OMNIC 3.2 software, where 128 scans were recorded with 4 cm^{-1} resolution. The polysaccharide was pressed into KBr pellet with a sample/KBr ratio 1/200 mg. NMR Spectra were measured in D_2O at 25 °C on Varian 600 MHz spectrometers: UNITY INOVA 600 NB and VNMRS, equipped with 5 mm multinuclear probe with inverse detection and 5 mm $1H\{^{13}C, ^{15}N\}$PFG Triple Res IDTG600-5 and equipped with HCN ^{13}C enhanced salt tolerant cold probe, respectively, both with *z*-gradients. Samples were freeze-dried from 95% D_2O and after they were dissolved in 99.98% D_2O. For both, ^1H and ^{13}C NMR spectra, chemical shifts were referenced to internal acetone (δ 2.217 and 31.07, respectively). Standard 1D and 2D pulse sequences from Varian pulse program library was used for signal assignments.

2.5. Antitussive activity assay

The guinea pigs were divided into three groups. "Negative control" group received pure *water* for injection in the dose 1 mL/kg b.w. orally. "Positive control" group received *codeine phosphate* in the dose 10 mg/kg b.w. orally. To the third group of animals water solutions of *Fagus* sulfated *4-O-methylglucuronoxylan* in the dose of 50 mg/kg b.w. were administrated the same way. Each tested groups consisted of 10 animals. Codeine, sulfated 4-*O*-methylglucuronoxylan and citric acid were dissolved in water for injection.

The guinea-pig was placed in a body plethysmograph box (HSE type 855, Hugo Sachs Elektronik, Germany) and restricted, so that the head protruded into the head chamber and the neck was sealed with a soft diaphragm. The cough reflex was induced by the aerosol of citric acid in a concentration 0.3 mol/L, generated by a jet nebulizer (PARI jet nebulizer, Paul Ritzau, Pari-Werk GmbH, Germany, output 5 L/s, particle mass median diameter of 1.2 μm), and delivered for 3 min to the head chamber of the plethysmograph. The number of coughs was evaluated on the basis of sudden enhancement of expiratory flow accompanied by a typical cough movement and sound observed by a trained staff, during the 3-min exposure to the tussigenic aerosol. The cough response was measured before administration of any agents (baseline measurement; *N* value in graphs) and then 0.5, 1, 2, and 5 h after their application. A minimal time interval between two measurements was 2 h to prevent of cough receptors against adaptation to the irritant, which could influence the response.

2.6. Specific airway resistance

Reactivity of airway smooth muscles *in vivo* was expressed as specific airway resistance calculated according Pennock et al. [19]. The value of specific airway resistance is proportional to the phase difference between nasal and thoracic respiratory airflows recorded in the head and thoracic chambers of the plethysmograph, respectively, which means that the bigger phase difference the higher the value of specific airway resistance and also a greater degree of bronchoconstriction [20]. The specific airway resistance was measured consecutively after the citric acid exposures and cough response registration during a 1 min interval. Their intensity before water, codeine phosphate and 4-*O*-methylglucuronoxylan applications were considered as baseline (*N* value in graphs). The next were measured in 0.5, 1, 2 and 5 h intervals.

2.7. Statistical analysis

Data were presented as mean ± standard error of the mean (SEM). Changes in the number of citric acid-induced cough efforts and in specific airway resistance from baseline after the consecutive time intervals (0.5, 1, 2, and 5 h) elapsing from administration of compounds were evaluated with a Student *t*-test. $p < 0.05$ was considered to indicate a significant difference.

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