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STUDY OF SPECIFIC PHARMACOLOGICAL ACTIVITY OF SODIUM SALT (4-O-B-GLUCOPYRANOSYLOXY)-BENZOIC ACID

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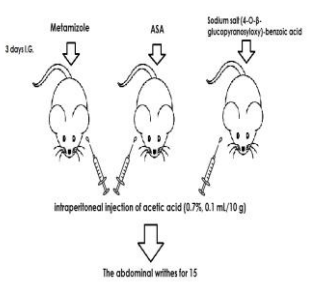
Graphical abstract



Abstract

Chronic inflammatory diseases of various genesis are prevalent today. Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used to treat pain and inflammation, but their long-term use is associated with complications in the gastrointestinal tract, including peptic ulcers. We synthesized a molecule of sodium salt (4-O-β-glucopyranosyloxy)-benzoic acid. This substance has diuretic and anti-inflammatory activities. It should be noted that most of NSAIDs has analgesic effect. In this connection, the aim of this study was to evaluate the analgesic activity of sodium salt (4-O-β-glucopyranosyloxy)-benzoic acid. We studied analgesic effect in the test "acetic writhing". Sodium salt (4-O-β-glucopyranosyloxy)-benzoic acid significantly reduces the number of writhing by 14 units during the experiment, as an alternative criterion percent of animals with analgesia was 42.6%. Thus, in the test "acetic writhing" revealed the presence of the analgesic activity have developed drug average severity.

Keywords: Pain, analgesic, phenolic glycosides, writhing test

1.0 INTRODUCTION

Chronic inflammatory diseases of various geneses are prevalent today. Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly used to treat pain and inflammation, but their long-term use is associated with complications in the gastrointestinal tract, including peptic ulcers [1]. NSAIDs efficiency validated in preclinical and clinical trials, as well as through clinical experience of doctors and patients. More than 80% of doctors all over the world prescribe NSAIDs. Two thirds of patients take these drugs for various inflammatory diseases including chronic inflammatory diseases of urinary tract, which usually accompanied by pain syndrome. Usually, the combination therapy is prescribed for inflammation of the urinary tract. It includes diuretics NSAIDs and antimicrobials. Combination therapy down regulates inflammation, urine volume and accelerated bacterial clearance [2]. But these drugs have serious side effects. However, we note that the treatment of the urinary tract is a considerable difficulty, most drugs used in therapy, such as antibiotics and NSAIDs are among the drugs possessing nephrotoxic. Also mentioned are other possible side effects.

Antimicrobial drugs violate the normal microflora of the digestive tract and others [3]. Assignable diuretic drugs also have side effects. They are primarily related to impairments of water-electrolyte balance, which limits its use in the treatment of chronic diseases of the kidneys and urinary system, as well as in various cardiovascular diseases [4]. In this regard, there is growing interest in herbal medicine.

The most often prescribed leaves of Vaccinium vitis-idaea and Arctostaphylos uva-ursi, buds of Betula pendula and flowers of Centaurea cyanus, grass Knotweed, Juniper and kidney tea [5, 6]. The advantage of herbal remedies combined four essential for the treatment of this group of disease pharmacological effects: mild diuretic, anti-inflammatory, antimicrobial and antioxidant action. Figure 1 shows the synthesis of sodium salt (4-O-β-glucopyranosyloxy)-benzoic acid. Reagents and conditions

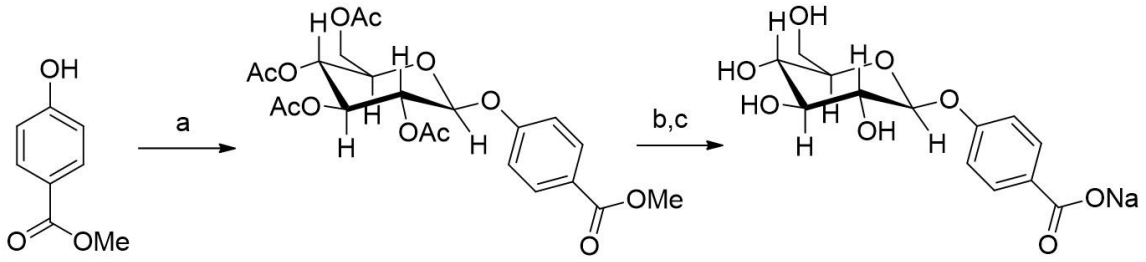


Figure 1 Synthesis of sodium salt (4-O-β-glucopyranosyloxy)-benzoic acid. Reagents and conditions: (a) β-pentaacetate glucose, $BF_3{\cdot}Et_2O$, TEA, chloroform, 20 h, rt; (b) MeOH, MeONa; (c) NaOH

Alternatives are plants that have these types of activity, for example plants containing arbutin. However, use of this group of plants also has some side effects, because arbutin is hydrolyzed to toxic metabolite hydroquinone. Cytotoxicity, mutagenicity, hepatotoxic, leukopenia belong to negative effects of a hydroquinone [7]. We synthesized a series of compounds based on the structure of arbutin, and which was chosen the most active. Sodium salt (4-O-β-glucopyranosyloxy)-benzoic acid was obtained follow Scheme 1. The structure and purity of all acetylated products were proven by complex of analysis (mp, IR, NMR and GH/MS). Deprotected glycosides were not analyzed by GH/MS. Glycosides configuration was defined by method described in [8]. Sodium salt (4-O-β-glucopyranosyloxy)-benzoic acid has diuretic and anti-inflammatory activities. It should be noted that most of NSAIDs has analgesic effect. In this connection, the aim of this study was to evaluate the analgesic activity of sodium salt (4-O-β-glucopyranosyloxy)-benzoic acid.

2.0 MATERIALS AND METHODS

2.1 Drugs and Pharmacological Treatments

The following drugs were used: acetylsalicylic acid (ASA) (Irbit's chemical-pharmaceutical factory, Russia), metamizol sodium (Moscow Pharmaceutical Factory, Russia).

2.2 Chemistry

Melting points, which are uncorrected, were determined using MP50 Melting point system (Mettler toledo). IR spectra were recorded with IR Fourier spectrophotometer Spectrum BX II using KBr disks. The ^1H and ^{13}C NMR spectra were recorded on Bruker-300 MMX spectrometer at 300 and 75.5 MHz, respectively, in DMSO-d6 and D_2O-d2 with TMS as an internal standard. The chemical shifts are given in d (parts per million) and the spin-spin coupling constants (J) in hertz. GC-MS analysis was performed using Agilent 7890A/5975C GC/MSD instrument, electron energy 70 eV. The ion source temperature was 230 °C, with the quadrupole temperature 150 °C and evaporator temperature 315 °C, employing a 30.000x0.25 mmx0.25 μm HP-5MS fused-silica capillary column. Helium was used as carrier gas at a constant flow of 1 mL/min and an inlet temperature of 315 °C. The column temperature mode: 2 min at 70 °C, 70-315 °C (10 °C/min), and 25 min at 315 °C. Chloroform was used after drying with P_2O_5.

2.2.1 1,2,3,4,6-penta-O-acetyl-β-D-glucose

Was obtained according to the method described in lit [9] and additionally recrystallized from ethanol. Yield 86%, mp 130 °C.

2.2.2 Methyl 4-(2,3,4,6-tetra-O-acetyl-β-D glucopyranosyloxy)benzoate

Was obtained according to the method [10] by glycosylation of methylparaben with boron trifluoride diethyl etherate in dry chloroform. Yield 73%; mp 156 °C;

2.2.3 Methyl 4-(β-D-glucopyranosyloxy) benzoate

Was obtained according to the method [10] by deprotection of methyl 4-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyloxy)benzoate with sodium methylate in methanol. Yield 96%; mp 167 °C;

2.2.3 Sodium salt (4-O-β-glucopyranosyloxy)-benzoic acid

To a solution of 1.07 g (3.4 mmol) Methyl 4-(β-D-glucopyranosyloxy) benzoate in 30 ml H_20 was added 0.19 g NaOH (3.5 mmol). The mixture was stirred 24 h under room temperature. Then solution was evaporated. Yield 98%; mp 273 °C; ^{1}H NMR (D_2O, 300 MHz,), δ: ; 3.42-3.80 (6H , m, H-2', H-3', H-4', H-5', H-6'b, H-6'a); 5.1 (1H, m, H-1'); 7.02 (2H, d, J=8.4 Hz, H-3, H-5); 7.74 (2H, d, H-2, H-6, J=8.4 Hz), ^{13}C NMR , (D_2O, 75.5 MHz), δ: 60.3 (CH2, C6'); 69.2 (CH, C-4'); 72.7 (CH, C-2'); 75.3(CH, C-5'), 75,9 (CH, C-3'); 99.5(CH, C-1'); 115.5(2×CH, C-2, C-6); 131.4(2×CH+C, C-3, C-5 + C-4); 158.5(C, C-1); 174.8(CO, CONa).

2.3. Animals

Young adults Swiss mice (23–25 g) were obtained from the Institute of Cytology and Genetics SB RAS, Novosibirsk, Russia. Animals were maintained at controlled room temperature with free access to food and water, under a 12 h light/dark cycle. All experimental procedures were performed in accordance with «European Convention for the Protection of Vertebrate Animals used for experimental and other scientific purposes» Strasbourg, (1986).

2.4 Acetic Acid-induced Abdominal Constriction Test

Abdominal writhes were induced by intraperitoneal (i.p.) injection of acetic acid (0.7%, 0.1 mL/10 g) in mice [11]. Animals were pretreated orally (p.o.) with sodium salt (4-O-β-glucopyranosyloxy)-benzoic acid (20 mg/kg), Metamizole sodium (20 mg/kg), acetylsalicylic acid (ASA,20 mg/kg), or vehicle (saline solution, 0.1 mL/ 10 g) for 3 days and 60 min before initiating the algesic stimulation (n=8/group). The abdominal writhes were observed for a period of 15 min and starts counting from the moment of the first cramps.

2.5 Statistical Analysis

For in vivo studies, the results were expressed as mean ± standard error means (S.E.M.). Statistical evaluation of the data was performed using one-way analysis of variance (ANOVA) followed by Bonferroni's test. Values of $P<0.05$ were considered statistically significant.

3.0 RESULTS

It was established that the number of writhings averaged 34,87 ± 3,2 in the control group. ASA was taken as the reference drug. It reduces the number of writhing by 2 units for 15 minutes on the alternative criterion the percent of animals with analgesia was 7.82%. Metamizole reduced the number of writhing not significantly and increased the latent time of pathological reactions to flogistik. Sodium salt (4-O-β-glucopyranosyloxy)-benzoic acid significantly reduces the number of writhing by 14 units during the experiment, as an alternative criterion percent of animals with analgesia was 42.6%. Thus, in the test "acetic writhing" revealed the presence of the analgesic activity have developed drug average severity. Table 1 shows the analgesic effect in acetic acid induced writhing model

Table 1 Analgesic effect in acetic acid induced writhing model

Group	№ of writhes	Latent development time s.	Inhibition %
Control (vehicle) 0,25 ml	34,87±3,2	214±9,0	
ASA 17 mg/kg	32,14±2,16	246±22,0	7,82
Metamizole 17 mg/kg	31,4±3,2	262±23,8	9,95
Sodium salt (4-O-β-glucopyranosyloxy)-benzoic acid 17 mg/kg	20,01±3,9**	314±39,0*	42,6

Values are expressed as mean ± standard error mean (n = 8).
*p < 0.05; ** p < 0.01 compared to the induced group.

4.0 DISCUSSION

The pathogenesis of the pain caused by inflammation or damage of tissues due to the irritation or sensitized nociceptive nerve ending to inflammatory mediators. Injured cells, blood vessels, stem cells, white blood cells are the source of these mediators [12]. Nonsteroidal anti-inflammatory drugs (NSAIDs) have the most pronounced effect on the peripheral components of pain - somatic and neurochemical [13]. In our experiment the injection of acetic acid produced hyperalgesia in the form of abdominal muscle contraction accompanied by an extension of the forelimbs and body elongation. Sodium salt (4-O-β-glucopyranosyloxy)-benzoic acid attenuated the abdominal constriction provoked by acetic acid, unlike reference drugs. Thus, one possible mechanism of the analgesic activity of developed compound could be due to the blockade of the effect or the release of endogenous substances (arachidonic acid metabolites) that excite pain nerve endings. In fact, in the mechanism of suppression of visceral and deep somatic pain as well as the spastic contraction of the abdominal muscles may be involved and other routes.

Different mechanisms may be involved in the reduction of muscular constriction; for instance, sympathetic system acts through the release of biogenic amines, cyclooxygenases, and their metabolites inhibition and through opioid receptor interaction [14]. In order to elucidate possible

mechanisms, it should be noted that acetic acid-induced writhing test and carrageenan-induced rat paw edema lacks specificity. Because these kinds of activities may not be directly related to the inhibition of the effect of pro-inflammatory mediators. Drug target interactions may be located at the level of transcription factors that play a crucial role in the expression of many genes involved in immune and inflammatory responses.

5.0 CONCLUSION

Presented results of demonstrate that sodium salt (4-O-β-glucopyranosyloxy)-benzoic acid has analgesic activity of an average degree of impact. Therefore, studies of other specific activities affecting the inflammation are relevant for understanding the mechanism of action.

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