

## NEW ANTIRHEUMATIC SUBSTANCE BASED ON PHENOLIC GLYCOSIDE STRUCTURE METHYL (4-O-B-GLUCOPYRANOSYLOXY)-BENZOIC ACID

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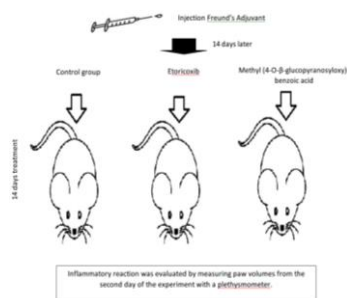
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### Graphical abstract



### Abstract

Rheumatoid arthritis (RA) is a chronic inflammatory multifactorial disorder affecting approximately 1% of population, with over 3000000 new cases annually. The principal pharmacological agents are nonsteroidal anti-inflammatory drugs (NSAID). Patients with rheumatoid arthritis are vulnerable to NSAID-associated gastrointestinal complications. Based on these findings we developed a new NSAID agent based on phenolic glycoside structure methyl (4-O-β-glucopyranosyloxy)-benzoic acid. In this study we evaluated the methyl (4-O-β-glucopyranosyloxy)-benzoic acid therapeutic effect in adjuvant-induced rat arthritis compared to etoricoxib effect. According to the results, methyl (4-O-β-glucopyranosyloxy)-benzoic acid activity was superior to etoricoxib, paw volume returned to the initial value 2 days early, than it did in etoricoxib group. Therefore, methyl (4-O-β-glucopyranosyloxy)-benzoic acid might contest to the modern antirheumatic drug etoricoxib.

Keywords: Rheumatoid arthritis, NSAID, phenolic glycosides, adjuvant arthritis

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## 1.0 INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory multifactorial disorder affecting approximately 1% of population, with over 3000000 new cases annually [19]. Individuals with rheumatoid arthritis have substantially shorter life expectancy that does the general population, so the significance of the disease cannot be overestimated [2]. RA also has been associated with genetic disorders and environmental risk factors, which prevent to create effective treatment approach [5].

At this time, RA treatments are focused on the reduction of pain, inflammation and joint damage. The principal pharmacological agents are nonsteroidal anti-inflammatory drugs (NSAID), disease-modifying antirheumatic drugs,

glucocorticoids, and specific inhibitors of the mediator response [3]. Not only positive, but also negative severe adverse effects of the treatment are known [10]. NSAID have been associated with gastrointestinal complications from NSAID-related peptic ulcer disease to gastrointestinal perforation, particularly in aged individuals [14]. Therefore, patients with rheumatoid arthritis are vulnerable to NSAID-associated gastrointestinal complications [4, 8, 10]. NSAID-induced gastrointestinal mucosal injury has been associated with endogenous prostaglandins deficiency, as a result of cyclooxygenase inhibition [21, 24].

Elevated level of NO synthase activity, bacterial flora and neutrocytosis are high impact factors of NSAID-related gastrointestinal complications [1, 11, 22, 23, 25]. Recent reports confirm the key role of

nitrous oxide in the pathogenesis of indomethacin-induced small-intestine ulceration in rat rheumatoid arthritis models. According to the literature sources, there is a straight correlation between endogenous nitrous oxide quantity and administered indomethacin dose in the experimental animals [7, 9, 16].

Based on these findings we developed a new NSAID agent based on phenolic glycoside structure methyl (4-O- $\beta$ -glucopyranosyloxy)-benzoic acid. Therapeutic effect of this substance should be superior to current antirheumatic agents. In this study we evaluated the methyl (4-O- $\beta$ -glucopyranosyloxy)-benzoic acid therapeutic effect in adjuvant-induced rat arthritis compared to etoricoxib effect.

## 2.0 MATERIALS AND METHODS

### 2.1 Drugs and Pharmacological Treatments

In the experimental rheumatoid arthritis model we used Freund's Adjuvant, Complete (Sigma F5881), methyl (4-O- $\beta$ -glucopyranosyloxy)-benzoic acid, etoricoxib (Arcoxia, Merck & Co. Inc. USA.), methyl (4-O- $\beta$ -glucopyranosyloxy)-benzoic acid Was obtained according to the method by glycosylation of methylparaben with boron trifluoride diethyl etherate in dry chloroform.

### 2.2 Experimental Animals

We used Wistar adult male rats weighing 200 to 220 g (8-12 weeks old). The animals were obtained from The Federal Research Center Institute of Cytology and Genetics of Siberian branch of The Russian Academy of Science, Novosibirsk, Russia. The rats were housed in the vivarium, allowed ad libitum access to rat chow and water, and were subjected to a natural photoperiod of 12-h light/dark cycle natural photoperiod of 12-h light/dark cycle (Fluorescent Lighting), with air temperature  $25 \pm 1^\circ\text{C}$  and relative humidity  $55 \pm 5\%$ . All animals were acclimated to housing conditions one week before the experiment. 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).

### 2.3 Arthritis Modelling

The rats were randomly assigned to three groups, which received: methyl (4-O- $\beta$ -glucopyranosyloxy)-benzoic acid 17 mg/kg, etoricoxib 17 mg/kg and control group orally administered equivalent volume of deionized water 2ml. Examined substances were suspended through 2ml of deionized water. The fourth group was intact, no manipulations were performed. To induce immune reaction we injected PA Adjuvant 100  $\mu\text{l}$  into right hind paw plantar aponeurosis [12]. Inflammatory reaction was evaluated by measuring

paw volumes from the second day of the experiment with a plethysmometer. The substances were administered from 14 to 28 day of experiment.

### 2.4 Statistical Analysis

For in vivo studies, the results were expressed as mean  $\pm$  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.

## 3.0 RESULTS

The development of edema in the experimental groups was physiologic and was not significantly different (Table 1.). Paw volume changes in the intact group had not been exceeded the statistical error throughout the experiment. The administration of the substances was started from the 14 day of experiment, affecting the swelling increment trend. The most significant changes were observed in the control group: at the 28 day of experiment paw volume increased to 177% of the initial volume. Table 1 shows various trends of the therapeutic effect. Etoricoxib held the paw volume increment at the 16 day of experiment and decreased it to 131% of the initial value. On the contrary, methyl (4-O- $\beta$ -glucopyranosyloxy)-benzoic acid features cumulative effect. The test substance didn't decrease the paw volume at the 16 day with the increment of 147% of initial value. At the 18 day of experiment edema in methyl (4-O- $\beta$ -glucopyranosyloxy)-benzoic acid group rapidly decreased to 23% making 123% of the initial volume, which is comparable to etoricoxib. The methyl (4-O- $\beta$ -glucopyranosyloxy)-benzoic acid therapeutic effect was superior to etoricoxib, due to full paw recovery at the 24 day. The initial paw volume value in etoricoxib group was observed only at 26 day.

**Table 1** Anti-inflammatory effect of etoricoxib and methyl (4-O- $\beta$ -glucopyranosyloxy)-benzoic acid in rat adjuvant arthritis models I

Day	Intact group	Control group	Etoricoxib	Methyl (4-O- $\beta$ -glucopyranosyloxy)-benzoic acid
0	0,65 $\pm$ 0,01	0,73 $\pm$ 0,07	0,77 $\pm$ 0,04	0,73 $\pm$ 0,04
2	0,64 $\pm$ 0,02	0,85 $\pm$ 0,05	0,91 $\pm$ 0,04*	0,84 $\pm$ 0,05*
4	0,64 $\pm$ 0,02	0,93 $\pm$ 0,06	0,94 $\pm$ 0,05*	0,86 $\pm$ 0,07*
6	0,65 $\pm$ 0,01	0,94 $\pm$ 0,1	0,96 $\pm$ 0,06*	0,79 $\pm$ 0,09
8	0,65 $\pm$ 0,01	0,98 $\pm$ 0,11	0,98 $\pm$ 0,06*	0,96 $\pm$ 0,1*
10	0,65 $\pm$ 0,01	0,99 $\pm$ 0,08	0,98 $\pm$ 0,07*	1,0 $\pm$ 0,07*
12	0,65 $\pm$ 0,01	1,00 $\pm$ 0,05	1,02 $\pm$ 0,06*	1,03 $\pm$ 0,1*
14	0,65 $\pm$ 0,01	1,03 $\pm$ 0,13	1,04 $\pm$ 0,07*	1,05 $\pm$ 0,1*
16	0,65 $\pm$ 0,01	1,05 $\pm$ 0,08	1,01 $\pm$ 0,07*	1,07 $\pm$ 0,1*
18	0,65 $\pm$ 0,01	1,10 $\pm$ 0,05	0,93 $\pm$ 0,1*#	0,9 $\pm$ 0,03*#
20	0,65 $\pm$ 0,01	1,20 $\pm$ 0,12	0,89 $\pm$ 0,06*#	0,89 $\pm$ 0,03*#
22	0,65 $\pm$ 0,01	1,25 $\pm$ 0,08	0,88 $\pm$ 0,04*#	0,83 $\pm$ 0,03*#
24	0,65 $\pm$ 0,01	1,27 $\pm$ 0,06	0,82 $\pm$ 0,06 #	0,74 $\pm$ 0,04#
26	0,65 $\pm$ 0,01	1,29 $\pm$ 0,08	0,78 $\pm$ 0,08 #	0,74 $\pm$ 0,03#
28	0,65 $\pm$ 0,01	1,29 $\pm$ 0,1	0,78 $\pm$ 0,6 #	0,73 $\pm$ 0,05#

Values are expressed as mean  $\pm$  standard error mean (n = 10).

Note. \* - significant comparing to initial paw volume, p<0,01.

# - significant comparing to control group, p<0,05.

## 4.0 DISCUSSION

In this research, we studied methyl (4-O- $\beta$ -glucopyranosyloxy)-benzoic acid antirheumatic activity in adjuvant arthritis model. Current RA reports emphasize the importance of early anti-inflammatory treatment in joint damage prevention [15]. High effectiveness and safety are vital aspects of modern pharmacotherapy and remain the basic criteria for new pharmacological agent selection. Due to various adverse effects, many new pharmaceutical agents stick at the level of preclinical and clinical trials. The complexity of new drugs finding hampers the development of modern pharmacology. Using low-toxicity and harmless substances in new structures design is one of the ways of «safe search» principle realization. The result of methyl (4-O- $\beta$ -glucopyranosyloxy)-benzoic acid hydrolysis is non-toxic glucose and Ethyl 4-hydroxybenzoate. Acute, subchronic, and chronic studies in rats indicate that parabens as pharmaceutical and cosmetic preservatives are practically non-toxic. Experimental studies have not reveal mutafacient or teratogenic effect in rat offspring. [20]. Reports show perspective and safety of methyl (4-O- $\beta$ -glucopyranosyloxy)-benzoic acid considering its non-hazardous metabolites. The current line of therapy typically involve a combination of several drugs. The complexity of RA pathogenesis determines combined and long treatment. Arthritis is a complex inflammatory process, chemotaxis- (IL-1, IL-6, IL-10, TNF-alpha, INF-gamma, adhesion factors) and WBC-mediated [18]. We know, that

etoricoxib is COX-2 selective inhibitor [13], but today we cannot be sure about methyl (4-O- $\beta$ -glucopyranosyloxy)-benzoic acid mechanism of action, because, as mentioned above, there various therapeutic mechanisms. The difference between methyl (4-O- $\beta$ -glucopyranosyloxy)-benzoic acid and etoricoxib time of action might be an indirect evidence of possible alternative pathway. Antirheumatic management includes not only NSAID, but methotrexate - an antimetabolite, structurally similar to folic acid. There are reports about severe adverse effects of wide used etoricoxib-methotrexate combination. Individuals developed Stevens-Johnson syndrome (SJS), a form of toxic epidermal necrolysis (TEN) [6, 17]. Due to its structure, methyl (4-O- $\beta$ -glucopyranosyloxy)-benzoic acid industrial synthesis is low-cost, few stage process, which doesn't require high-tech equipment. High effectivity, low cost and low toxicity of the new drug are the most important aspects on consumer market. We suppose it isnecessary to look for new pharmacological substances without negative side effects. One of these substances might be methyl (4-O- $\beta$ -glucopyranosyloxy)-benzoic acid.

## 5.0 CONCLUSION

The results of the present study reveal methyl (4-O- $\beta$ -glucopyranosyloxy)-benzoic acid substantial antirheumatic activity, therefore it considered to be a perspective substance for further research of it anti-inflammatory effect.

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