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EFFECTIVENESS OF THE ANTIVIRAL DRUG RUTAN ON THE COURSE OF ADJUVANT-INDUCED ARTHRITIS.

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ABSTRACT

Objectives. This study aims to investigate the impact of Rutan, a polyphenolic compound with high antiviral and anti-inflammatory activity, on adjuvant arthritis.

Methods. Experimental studies were conducted on sexually mature white rats to develop a model of adjuvant-induced arthritis in laboratory animals. The study involved injecting 0.1 ml of complete Freund's adjuvant (CFA) into the hind paw of the rats. The aim was to study the prophylactic and therapeutic effect of different doses of Rutan and diclofenac sodium. The paw edema was measured before and after the drug administration, and blood samples were collected to identify pro- and anti-inflammatory interleukins, tumor necrosis factor, and C-reactive protein.

Results. Rutan has shown anti-inflammatory activity (AIA) with prophylactic application, resulting in 27.5%, 30.9%, and 34.8% AIA on days 7, 10, and 14, respectively. The therapeutic application resulted in AIA of 24.1% on day 28 compared to the control group. Prophylactic use of Rutan and diclofenac sodium reduced IL-1 levels by 77.3% and 71.4%, TNFα by 69.9% and 73.6%, and CRP by 77.1% and 80.5%, while increasing IL-10 levels by 64.5% and 75.2%, respectively. Rutan and diclofenac treatment group showed similar results, with IL-1 levels decreasing by 74.6% and 79.8%, TNFα by 65.5% and 70.3%, and CRP by 83.8% and 87.4%, while increasing IL-10 levels by 48.3% and 69.0%, respectively.

Conclusions. Rutan shows strong pharmacological activity in inhibiting inflammation in rats with arthritis, comparable to diclofenac sodium.

Keywords

adjuvant-induced arthritis, cytokines, prevention, treatment, Rutan

Introduction. Treatment of a widespread pathology - rheumatoid arthritis (RA) requires improvement of methods and means of pharmacotherapy since the used therapeutic agents usually cause the development of a significant number of side effects, sometimes life-threatening complications: gastro-, nephro-, cardio-, hemato-, hepatotoxicity, which is associated with their ability to penetrate histohematic barriers and the appearance of systemic action [1, 2, 3, 4, 5, 6, 7]. The

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progress achieved in the treatment of RA in recent years is associated, on the one hand, with the improvement of methods for early diagnosis of the disease, which makes it possible to begin thorough therapy with basic anti-inflammatory drugs, and, on the other hand, with the development of a new class of drugs - genetically engineered biological drugs that block the leading links in the immunopathogenesis of diseases [8.9]. However, despite the fact that they allowed to increase the effectiveness of therapy and improve the prognosis in patients suffering from the most severe forms of RA led to a drastic increase in the cost of treatment [10]. However, despite the fact that they have made it possible to increase the effectiveness of therapy and improve the prognosis of patients suffering from the most severe forms of RA, they have led to a dramatic increase in the cost of treatment [10]. Based on this, it seems important to use low-toxic and, in the biological aspect, active agents. In this regard, the use of medicines obtained from medicinal plants containing polyphenolic compounds seems promising. Previously, we demonstrated the high antiphlogogenic activity of chemical compounds containing polyphenols [11,12,13]. Along with these, the drug Rutan - a polyphenolic compound with high antiviral activity was synthesized and introduced by the scientists of the Institute of Bioorganic Chemistry of the Academy of Sciences of the Republic of Uzbekistan [14,15]. Experimental studies have shown that this drug has distinct anti-inflammatory activity [16,17]. However, the effect of this drug on the course of adjuvant arthritis, which is the classic model of rheumatoid arthritis found in humans, has not been studied.

The purpose of this work was to study the influence of Rutan on the course of adjuvant arthritis in preventive and therapeutic applications.

Materials and research methods

All experimental studies were conducted on sexually mature white rats - males with an initial weight of 165-180 g, a total of 36 individuals obtained from the vivarium of the Department of Sanitary and Epidemiological Surveillance of the Main Medical Directorate under the Administration of the President of the Republic of Uzbekistan. Prior to the start of the experiment, all animals were examined and weighed after a two-week quarantine. Age, sex, physical activity and skin condition of animals were taken into account. Each experimental and control group consisted of six individuals. During the entire period of preparation for the experiment, during its conduct, laboratory animals were kept in a vivarium at a temperature of 20-25 ° C, humidity of at least 50%, in a well-ventilated room and day/night light mode, in plastic cages of 6 animals each, with a standard diet. The experiments were carried out in accordance with the “Rules for carrying out work using experimental animals”, as well as the rules adopted in the European

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Convention for the Protection of Vertebrate Animals Used for Experimental Research or for Other Scientific Purposes (ETS No. 123, Strasbourg, 03/18/1986).

A model of adjuvant-induced arthritis in laboratory animals was developed by intradermal injection into the dorsal surface of the hind paw of 0.1 ml of complete Freund's adjuvant (CFA) (Chondrex, Inc., USA), which contains killed mycobacteria H37RA at a concentration of 2 mg/ml suspended in oil intended to reproduce adjuvant-induced arthritis in rats [18]. Edema of the hind paw was controlled from the first to the 14th day of the experiment. When studying the prophylactic effect of drugs after CFA injection, the animals were divided into three groups of 6 animals each. The first and second groups received a daily intragastric injection of Rutan and diclofenac sodium at doses of 25mg/kg and 10mg/kg respectively, for a period of 14 days. The third group served as the control group. In a separate set of experiments, the therapeutic effect of the same drugs was studied. Again, 18 animals were used and divided into three groups of 6 animals each. The first and second groups received intragastric injections of Rutan and diclofenac sodium in doses of 25mg/kg and 10mg/kg respectively, starting from day 15 and continuing for 28 days after AF injection. The third group served as the control group.The oncometric measurements of the affected paws were carried out using a plethysmometer before and, 7, 10, 14, 21 and 28 days after the administration of the drugs (Ugo Basile Srl, Italy). The anti-inflammatory activity (AIA) value of the drugs was calculated using the formula:

$$\mathbf{AIA=V_{control}-V_{drug}/V_{control} \times 100 = \%.}$$

Where, $\mathbf{V_{control}}$ - average increase in paw volume in the control group, cm3,

$\mathbf{V_{drug}}$ –average increase in paw volume in experimental group, cm3.

One day after the last manipulation, blood was taken from the tail vein for hematological and biochemical studies. In blood serum samples, the levels of pro- (IL-1β) and anti-inflammatory (IL-10) interleukins, tumor necrosis factor (TNF-α) and C-reactive protein (CRP) were determined using an AT-858 enzyme immunoassay analyzer from Shenzhen Mindray Bio. Medical using commercial ELISA kits produced by Human Diagnostics and Vector-Best (Russia).

The data obtained were processed by the method of variation statistics using the paired Student's test and one-way analysis of variance using the standard software package BIOSTAT 2009 with an assessment of the significance of indicators (Mean±Std error). Differences in the compared groups were considered significant at a significance level of 95% $p < 0.05$.

Results and discussion

Rheumatoid arthritis (RA) is characterized not only by chronic erosive arthritis, but also by systemic damage to internal organs, leading to disability and, as a consequence, a reduction in the life expectancy of patients [19]. This pathology

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is more often diagnosed in elderly patients, for whom the use of nonsteroidal anti-inflammatory drugs is important [20]. Chronic joint inflammation induced by Freud's complete adjuvant is a classic experimental animal model of human's RA [21,22]. The results of experimental studies showed that injection of CFA leads to the development of a pronounced inflammatory process. A lethargy, aggressiveness, decreased mobility, dull hair, and decreased food intake was observed in rats starting from the third day after immunization. Moreover, a significant increase in inflammation was noted after 7, 10 and 14 days from the start of the experiment, and the volume of the rats' paws was more by 314.9, 328.3 and 343.2%, respectively, compared to the initial paw volume (Table 1). Some increase in volume was also noted in other joints. All this indicates the development of chronic progressive generalized immune-mediated inflammation in the joints. In contrast, in the group of rats that received Rutan preventatively, the increase in paw volume was less pronounced.

Table 1. The effect of Rutan and diclofenac sodium on the course of adjuvant-induced arthritis in preventive application (M±m, n=6)

Groups	Paw volume, cm^3 (days)			
	Initial	7	10	14
Control	0,67 ± 0,02	2,79 ± 0,04*	2,87 ± 0,05*	2,97 ± 0,06*
Rutan	0,68 ± 0,02	2,20 ± 0,03*	2,19 ± 0,03*	2,17 ± 0,04*
Diclofenac	0,62 ± 0,02	2,17 ± 0,10*	2,13 ± 0,08*	2,16 ± 0,03*

Note: * - *significant difference with the initial indicators of the corresponding groups of animals.*

Thus, the index of inflammation relief under the influence of Rutan at the specified time of the experiment was 27.5, 30.9 and 34.8%, respectively. A similar pharmacotherapeutic effect was noted in the group of rats receiving diclofenac sodium. During the specified observation periods, the index of inflammation suppression was 28.0, 32.7 and 34.3%, respectively. The obtained results showed that Rutan had an inhibitory effect on the development of adjuvant arthritis and had almost the same preventive effect as diclofenac sodium.

Most patients seek medical help after the development of a pathological condition in which the use of medicines becomes therapeutic application. Considering this condition, it seemed important to study the effect of Rutan on the treatment of adjuvant-induced arthritis when it was used therapeutically. The studied drugs were administered starting from the 15th day from the start of the CFA injection. As can be seen from the data in table 2, the volume of the paws of the experimental animals was clearly preserved for the next two weeks. Thus, compared to the initial volume of paw, two weeks after the CFA injection, the volume of the paws was increased by 338.2%, and after three and four weeks, by

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345.6 and 357.3%, respectively. In contrast, in the group of animals receiving diclofenac sodium and Rutan it was clearly less. We observed the most pronounced effect on the 28th day of the experiment, where the decrease was by 23.5 and 24.1%, respectively compared to the control group.

Table 2. Study of the therapeutic effect of Rutan and diclofenac sodium on the course of adjuvant-induced arthritis (M±m, n=6).

Groups	Paw volume, cm^3 (days)			
	Initial	14	21	28
Control	0,68 ± 0,02	2,98 ± 0,06*	3,03 ± 0,07*	3,11 ± 0,08*
Rutan	0,67 ± 0,02	2,88 ± 0,04*	2,65 ± 0,05*	2,36 ± 0,06*
Diclofenac	0,62 ± 0,02	2,84 ± 0,03*	2,55 ± 0,04*	2,38 ± 0,05*

Note: ** - significant difference with the initial indicators of the corresponding groups of animals.*

During this observation period, the anti-inflammatory activity of the drug was 27.6 and 30.4%.

Consequently, the use of Rutan has an inhibitory effect on the course of AIA and its activity is practically not inferior to the classic nonsteroidal anti-inflammatory drug - diclofenac sodium.

In clinical condition, in terms of assessing the effectiveness of therapeutic treatment, diagnostic tests are used to assess the severity of the inflammatory process. In this regard, the conducted biochemical studies showed that in the control group, the level of IL-1, TNFα and CRP increased by 4.6, 3.2 and 8.1 times against the background of a decrease in the level of IL-10 by 20.5% compared to the intact group on the 14th day of the experiment.

In animals receiving Rutan and diclofenac sodium, the degree of change in the studied parameters was significantly less. Thus, in the group of animals receiving these drugs preventatively, the level of IL-1 was reduced by 77.3% and 71.4%, TNFα - by 69.9 and 73.6%, and CRP by 77.1 and 80. 5%, respectively (Table 3). In contrast, the level of IL-10 exceeded the control level by 64.5 and 75.2%. We noted almost the same direction of change in other groups of animals that received the studied drugs for therapeutic purposes. As can be seen from the data in table 3, under the influence of Rutan and diclofenac sodium, the level of IL-1 decreased by 74.6% and 79.8%, respectively, TNFα - by 65.5 and 70.3%, and CRP - by 83.8% and 87.4%. These changes were accompanied by an increase in IL-10 levels compared to the untreated group by 48.3% and 69.0%, respectively. It should be noted that the values of control animals remained almost unchanged, as on the 14th day of research.

Consequently, reducing the intensity of the chronic inflammatory process in AIA is accompanied by a significant increase in the level of pro-inflammatory

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interleukins, against the background of a decrease in anti-inflammatory interleukins, both with the preventive and therapeutic use of Rutan and diclofenac sodium. It is noteworthy that these changes were accompanied by the reduction of the integral indicator of inflammation – the level of CRP. It should be noted that the pharmacotherapeutic activity of Rutan was not significantly inferior to diclofenac sodium. It is important that Rutan, being an antiviral drug, also has antiphlogogenic activity, including in such pathologies as autoimmune adjuvant arthritis, which is its advantage over other nonsteroidal anti-inflammatory drugs. This is probably due to the fact that Rutan is an interferon inducer [14,15], and an increase in the concentration of interferons, as a rule, provides a regulatory effect on immunity [24]. The results of this work allow us to recommend Rutan as an effective anti-inflammatory agent, both in acute and chronic diseases in the pathogenesis of which inflammation plays a central role.

Table 3. The content of cytokines IL-1β, IL-10 and TNF-α, as well as CRP in the peripheral blood of rats with adjuvant-induced arthritis in the therapeutic and preventative effect of Rutan and diclofenac sodium (M±m, n=6)

indexes / groups	IL-1β, pg/ml	IL-10, pg/ml	TNF-α, pg/ml	CRP mg/l
Preventative effect				
Intact	2,10 ± 0,07	3,91 ± 0,11	1,65 ± 0,05	1,03 ± 0,03
Control	11,82 ± 0,46*	3,11 ± 0,09*	6,93 ± 0,39*	9,40 ± 0,43*
Rutan	2,81 ± 0,07*,#	5,13 ± 0,17*,#	2,08 ± 0,04*,#	2,15 ± 0,05*,#
Diclofenac	2,21± 0,07#	5,43 ± 0,19*,#	1,82 ± 0,08#	1,83 ± 0,03*,#
Therapeutic effect				
Control	13,40 ± 0,43*	2,92 ± 0,08*	7,30 ± 0,33*	9,70 ± 0,44*
Rutan	3,42 ± 0,09*,#	4,30 ± 0,14#	2,52 ± 0,04*,#	1,57 ± 0,03*,#
Diclofenac	2,70 ± 0,07*,#	4,90 ± 0,16*,#	2,17 ± 0,11*,#	1,22 ± 0,09#

Note: * - *significant difference compared to intact animals,* # - *significant difference compared to the control of the corresponding research periods.*

Conclusions

1. The antiviral drug clearly suppresses the intensity of chronic artiritis induced by Freund's adjuvant in experimental animals.

2. The drug Rutan by its pharmacotherapeutic activity is not inferior to the reference nonsteroidal anti-inflammatory drug - diclofenac sodium.

2. Rutan is not inferior to the standard nonsteroidal anti-inflammatory drug - diclofenac sodium by its pharmacotherapeutic activity.

3. The mechanism of Rutan's anti-inflammatory activity is likely due to its regulating effect on the level of interleukins, as well as a decrease in the degree of oxidative stress.

4. Rutan can be recommended as a pathogenetic medicine for the treatment of diseases in the pathogenesis of which inflammation plays a leading role.

Conflicts of interest

The authors declare no conflicts of interest.

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