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The study of the anti-inflammatory activity of Eforan.

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ABSTRACT

Searching for new effective and low-toxic antiphlogistics among the new classes of compounds that could have a selective and prolonged effect and minimum side effects is highly relevant. In this respect, the promising compounds are di- and monophosphonic compounds, which representative is a drug Dimephosphon. Objective of our study was to investigate the influence of Eforan, a new phosphonate, on various models of experimental inflammation, as well as on the development of edema caused by various mediators and modulators of inflammation. In the experiments on rats, Eforan reduced dose-dependently the intensity of the inflammatory reaction caused by carrageenan. The duration of anti-inflammatory action of Eforan was up to 24 hours of observation. Eforan effect on the carrageenan model of inflammation was comparable in the strength of anti-inflammatory effect with acetylsalicylic acid, and differed in longer duration of action. The anti-inflammatory effect of Eforan was demonstrated on the model of chronic autoimmune diseases - adjuvant arthritis. Both anti-histamine and anti-serotonin effects of the drug, as well as the absence of antagonism with bradykinin were established. The paper discusses the originality of the mechanism of anti-inflammatory action of phosphonates that show no gastrotoxicity unlike the traditional NSAIDs. It can be assumed that the anti-inflammatory effect of phosphonates, including Eforan, exemplified in cyclooxygenase-2 (COG-2) dependent inflammation models, is associated with the selective inhibition of COG-2.

Keywords: non-steroidal anti-inflammatory drugs, phosphonates, Eforan, inflammation, arthritis, carrageenan, histamine, bradykinin, Freund's adjuvant.

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INTRODUCTION

Disadvantages of the existing non-steroidal anti-inflammatory drugs (NSAIDs) and the increasing requirements of modern therapy thereto make it urgent to search for new effective and low-toxic anti-phlogistics among the new classes of compounds that could have a selective and prolonged effect and minimum side effects. In this respect, the promising compounds are di- and monophosphonic compounds, represented by Dimephosphon, which anti-inflamatory activity has been experimentally confirmed and found its practical application in the clinical practice [3,4]. The result of the analysis of the relationship of chemical structure and phlogotropic activity of the substituted phosphonates was a directed synthesis of a new compound - 2-carbobutoxypropyl phosphonic acid dibutyl ether, called Eforan [1]. Eforan relates to low-toxic compounds, as its LD50 value upon internal action on rats is more than 5000 mg/kg [1].

Objective of the study was to investigate the effect of Eforan on various models of experimental inflammation, as well as on the development of edema caused by various mediators and modulators of inflammation.

RESEARCH METHODS

The experiments were conducted on 370 outbred male and female white rats of 180-250 g, using the conventional models of inflammation [2,8]. Carrageenan 1% (Sigma) and a Freund adjuvant (composition - 1 part of lanolin, 2 parts of liquid paraffin, BCG dose of 5 mg/kg) were taken as phlogogenic agents, the inflammation mediators and modulators were histamine-based solution 0.1% (Sigma), serotonin creatine sulfate solution 0.01% (Sigma) and of bradykinin-triacetate solution 0.01% (Sigma), which were administered subplantarly in the left hind paw each of 0.1 ml. The size of edema was determined with Ugo Basile plethysmometer (Italy) [11] based on the difference in paw volume before and after administration of proinflammatory agents. Eforan was synthesized at A.E. Arbuzov Institute of Organic and Physical Chemistry, KRC RAS, Kazan [1]. A single dose of drug was administered intraperitoneally in the form of an aqueous suspension 15 minutes prior to the model reproduction. Control animals were administered intraperitoneally with an appropriate amount of distilled water. In the experiments with carrageenan-induced edema, Eforan was used at doses of 21 mg/kg, 42 mg/kg, 84 mg/kg, 168 mg/kg, and 336 mg/kg, and the size of edema was assessed at the time of its maximum development - 3 hours. To study the effect of the drug on the dynamics of development of carrageenan-induced edema, Eforan was administered at doses of 33.6 mg/kg, 100 mg/kg, 336 mg/kg, and edematous response was recorded 1, 2, 3, 18 and 24 hours after injection of a phlogogenic agent. As reference agent, acetylsalicylic acid (ASA) at a dose of 100 mg/kg was administered intraperitoneally. The size of edema, caused by histamine, serotonin and bradykinin, was recorded 15, 45 and 90 min after subplantar injection. To evaluate the effect of Eforan on adjuvant arthritis (AA), the drug was daily administered to the animals at a dose of 33.6 mg/kg after administration of Freund's adjuvant, and the size of edema was recorded on day 5, 15, 20, 23, 31 of the study. The intensity of secondary arthritis was assessed by the change in the volume of the contralateral (right) paw of rats. Studies were carried out in accordance with the rules of good laboratory practice (GLP) for preclinical research in the Russian Federation [15], as well as the rules and recommendations of the International European Convention for the Protection of Vertebrate Animals used in experimental studies [16]. The study was approved by Local Ethics Committee.

Data from all experiments were statistically processed using the Student's t-test and presented as $M \pm m$ (M - average value, m - standard error of the mean). Differences were considered significant at a probability level of 95% or greater ($p \leq 0.05$).

RESULTS AND DISCUSSION

Subplantar injection of carrageenan by the 3rd hour of the inflammatory reaction caused an increase in the volume of the rats' paw by 81% as compared to its original size. Eforan impaired the development of carrageenan edema 3 hours after the model reconstruction in all doses tested, subject to that the anti-inflammatory effect was dose-dependent. Thus, a dose of 21 mg/kg reduced the increase in rats' paw volume by 26% in comparison with the control, 42 mg/kg - by 20%, 84 mg/kg - by 35%, 168 mg/kg - by 54%, and 336 mg/kg - by 81.5% (Figure 1). Acetylsalicylic acid inhibited the intensity of the edematous response by 53%, which corresponds to the published data [8, 9]. The study of the effect of Eforan on the dynamics of carrageenan-induced edema found that the drug at a dose of 33.6 mg/kg, 18 hours after injection, inhibited the development of edema by 48%, after 24 hours - by 31%. Anti-inflammatory effect of the drug at a dose of

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100 mg/kg 3 hour after injection was 27%, after 18 hours - 45%, after 24 hours - 34%. 2 hours after administration of the phlogogenic agent at a dose of 336 mg/kg the intensity of the edematous reaction was lower than in the control by 64.5%, after 3 hours - by 58%, after 18 hours - 67%, and after 24 hours - 63%. ASA inhibited the edematous response induced by carrageenan 1 hour after administration by 69%, after 2 hours - by 63%, and after 3 hours - by 46%, without affecting its intensity after 18 and 24 hours of administration (Table 1).

Table 1. The intensity of rat paw edema caused by carrageenan (1%), with administration of Eforan and acetylsalicylic acid (ASA) in dynamics (increase in paw volume in % from the original, M±m, n=10)

Groups	Time, hours				
	1	2	3	18	24
Control (distil. water)	60+9	120+8	131+7	92+9	59+4
33.6 mg/kg % of edema depression	55+9 8	108+10 10	112+15 15	48+7* 48	40.5+7* 31
100 mg/kg % of edema depression	58+10 3	98.6+10 18	96+7* 27	51+5* 45	39+6* 34
336 mg/kg % of edema depression	43+6 28	42.6+4* 65	55+6* 58	30+4* 67	22+4* 63
ASA 100 mg/kg % of edema depression	19±9* 69	44±1* 63	71±10* 46	67±12 27	42±8 29

Note: * - P < 0.05 as compared with control.

Table 2. The intensity of rat paw edema caused by the inflammation mediators, with administration of Eforan at a dose of 336 mg/kg (increase in paw volume in % from the original, M+m, n=10)

Groups	Time, min		
	15	45	90
C Histamine E % of edema depression	38+5 13+2* 66	28+5 6+1* 79	13+3.5 1.6+1* 88
C Serotonin E % of edema depression	37+4 13+5* 65	57.6+6 18+7* 69	45+5 7+4* 84
C Bradykinin E % of edema depression	18+3 11.5+6 36	6.5+3 7+4 0	12+3 6.6+4 42

Note: * - P < 0.05 as compared with control, C - control (distilled water), E - experimental (Eforan).

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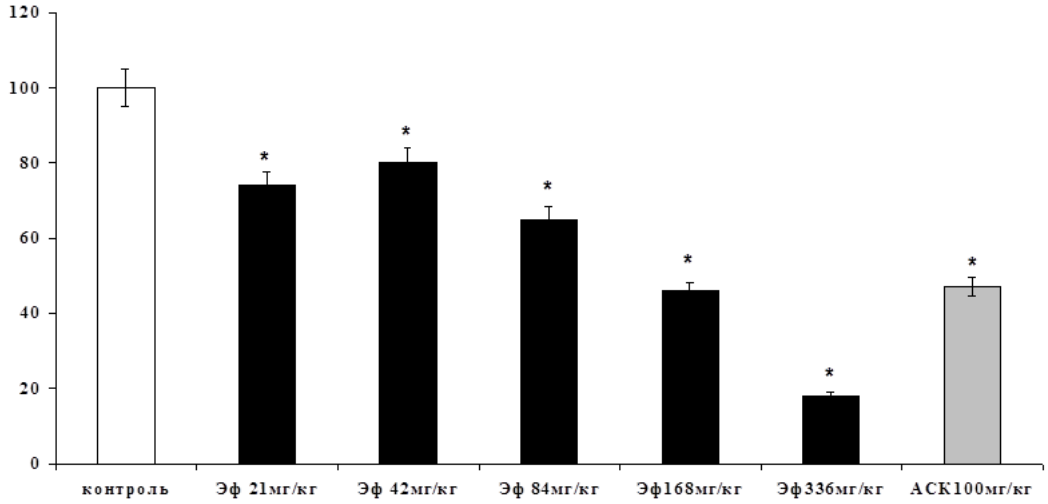
Fig. 1. The intensity of rat paw edema 3 hours after carrageenan administration (1%), with administration of Eforan (Ef) and acetylsalicylic acid (ASA). Y-axis - % of increase in paw volume, * - P<0.05.



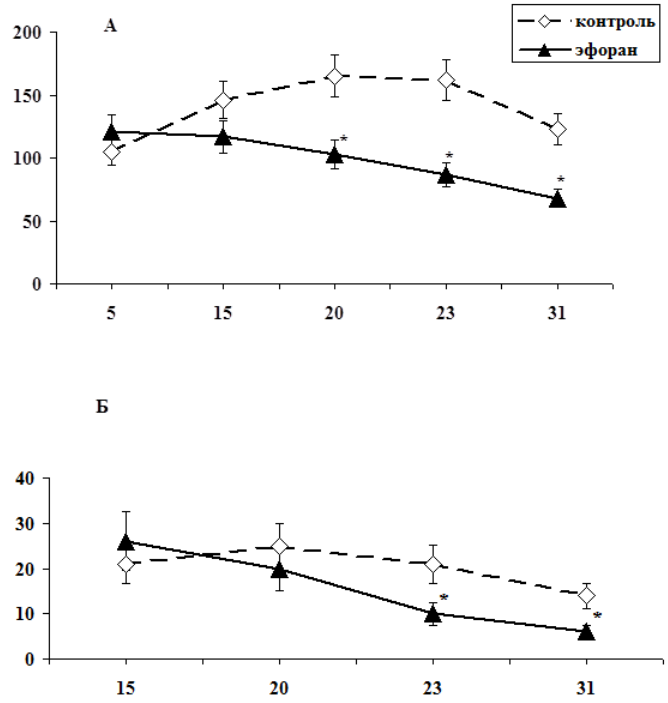


Fig. 2. The development intensity of primary (A) and secondary (B) arthritis upon subplantar administration of Freund's adjuvant with intraperitoneal administration of Eforan at a dose of 33.6 mg/kg. Y-axis - % of increase in paw volume, X-axis - recording periods, in days. * - P<0.05.

Thus, Eforan reduced the intensity of the inflammatory reaction caused by carrageenan, at the same time, the anti-inflammatory activity of the drug was dose-dependent - the higher the dose was, the earlier and more suppressed swelling of paws was. Anti-inflammatory effect of Eforan can be comparable with ASA: ED50 calculated graphically for Eforan was 0.45 mmol/kg (151 mg/kg), for ASA - 0.56 mmol/kg (100 mg/kg) [8,9]. The duration of anti-inflammatory effect of Eforan was up to 24 hours of observation, as opposed to an ASA, which effect lasted for 3 hours only. According to most authors, it is a model of acute inflammatory edema caused by carrageenan that allows for objective evaluation of anti-exudative effects of drugs and predicts their clinical efficacy [8, 12].

The analysis of the impact of Eforan to edemas caused by inflammatory mediators has established its anti-histamine, anti-serotonin activity and the absence of antagonism with bradykinin. (Table 2).

On day 2 of AA simulation, all animals developed an inflammatory response at the site of adjuvant injection, in the form of erythema and edema. At the end of the latent period on day 15 since the introduction of adjuvant, 80-90% of the animals had swelling of other limbs developed. Therapeutic and prophylactic administration of Eforan at a dose of 33.6 mg/kg helped to reduce swelling of the left paw of rats on day 20 of the study by 38%, on day 23 - by 46%, on day 31 - by 45%. The drug reduced the intensity of the secondary inflammation of the rats' right paw by 52.4% and 57% on day 23 and 31 of the study, respectively (Figure 2).

Thus, it was found that Eforan is effective for treatment of both acute arthritis and chronic inflammation similar to rheumatoid arthritis.

It is known that the mechanism of proinflammatory action of carrageenan is associated with cyclooxygenase-2 (COG-2) - COG isoenzyme [13], which in contrast to the constitutional COG-1 is expressed in cells and tissues during the development of the inflammatory response [14].

AA in rats was simulated as an autoimmune disease induced by subcutaneous injection of mycobacteria, which adequately reflects the pathological changes occurring in rheumatoid arthritis in humans, and is therefore valuable for the search for anti-inflammatory drugs [7]. In the mechanism of development of generalized inflammatory reaction in AA, as well as in rheumatoid arthritis in humans, COG-2 plays a key role by promoting the synthesis of prostaglandins in the inflammatory site [10].

Search for anti-inflammatory drugs, nongastrotoxic due to selective inhibition of COG-2, is a promising area of experimental pharmacology [10, 13, 14]. According to the literature, the phosphonates, in particular Dimephosphone and Mephoprane, also characterized by their anti-inflammatory effect on the model of inflammation caused by carrageenan, anti-histamine and anti-serotonin activity and by the absence of antagonism with bradykinin [6], which suggests a single mechanism of action of these drugs. It is important to note that Eforan in equimolar doses 3-4 times surpasses the already investigated analogues in its anti-inflammatory effect. In addition, one of the analogs of Eforana - Dimephosphone - reduces the damaging effect of Diclofenac, Indomethacin and acetylsalicylic acid on gastric mucosa [5]. Apparently, the phosphonates differ fundamentally from conventional non-steroidal antiphlogistics, and possibly may influence the regulation of the activity of the arachidonic acid cascade. It can be assumed that the anti-inflammatory effect of phosphonates, including Eforan, exemplified on COG-2 dependent models of inflammation, is associated with the selective inhibition of COG-2.

Probably, the anti-histamine and anti-serotonin activity of the drug is one of the factors that determine its anti-edematous effect, and contribute to the overall anti-inflammatory activity of Eforan.

SUMMARY

- Eforan has a dose-dependent anti-inflammatory effect on acute carrageenan-induced edema model in rats. Anti-inflammatory effect of Eforan is comparable in its strength to that of ASA, and has a more prolonged duration of action. The implementation of the anti-inflammatory action of Eforan involves also anti-histamine and anti-serotonin effects.
- Therapeutic and preventive action of Eforan inhibits the development of arthritis in rats caused by subplantar administration of Freund's adjuvant.

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