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RESEARCH ARTICLE

Influence of the potential dependent calcium channel blockers to the development of carrageenan-induced aseptic inflammation

Ziyovuddin Zaynutdinovich Khakimov[1], Alisher Khudayberdievich Rakhmanov[2], Nodira Bakhadirovna Bekova[1], Kodir Shukurlaevich Shukurlaev[2]

[1]Laboratory of Pharmaco-Toxicological Researches, Tashkent Medical Academy, Tashkent, Uzbekistan, [2]Departement of Pharmacology, Urgench Branch of the Tashkent Medical Academy, Tashkent, Uzbekistan

Correspondence to: Alisher Khudayberdievich Rakhmanov, E-mail: dr.ali.fl@mail.ru

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ABSTRACT

Background: A number of patients with diseases of the cardiovascular system simultaneously take anti-inflammatory medicines due to the presence of concomitant chronic inflammatory diseases such as rheumatism, arthritis, and podagra. Considering the above, it seems important to study the effect of calcium antagonists (CA) on the course of the inflammatory process. **Aim and Objectives:** The purpose of the study was an investigation of the effect of amlodipine, diltiazem, and cinnarizine in comparison with diclofenac sodium on the course of carrageenan-induced aseptic arthritis. **Materials and Methods:** The experiments were carried out on outbred white rats, males, and weigh 150–170 g. The inflammation was induced by the subplantary injection of a 1% aqueous solution of carrageenan in a volume of 0.1 ml. The studied medicines diclofenac sodium at a dose of 10 mg/kg, amlodipine and diltiazem at doses of 20 mg/kg, and cinnarizine at doses of 50 mg/kg were intragastrically administered to experimental animals 1 h before the injection of carrageenan. Measurement of the paw volume of animals was carried out using a plethysmometer **Results:** The anti-inflammatory activity of amlodipine in the indicated hours of observation was 34.1, 36.8, 37.8, and 39.7%, respectively. Cinnarizine also had a distinct anti-exudative effect, after 1, 2, 3, and 4 h of the experiment value of anti-inflammatory activity of it were as following: 38.6, 40.3, 41.9, and 44.0%, respectively. **Conclusion:** Blockers of potential-dependent calcium channels clearly suppress the exudative phase of aseptic inflammation. The studied medicines are arranged in a descending order by their pharmacological activity in the following row: diclofenac sodium = diltiazem> cinnarizine> amlodipine.

KEY WORDS: Inflammation; Carrageenan; Calcium antagonists

INTRODUCTION

The undoubted urgency of the problem of treating patients with arterial hypertension (AH) is annually confirmed by the formidable statistics of cardiovascular complications throughout the world. Despite the large arsenal of antihypertensive drugs, the choice of drugs for each individual patient is quite difficult. Antihypertensive drugs from the group of calcium antagonists (CA) are widely used by doctors. In international and domestic recommendations, CA are indicated as "first line" medicines for the treatment of hypertension along with beta-blockers, thiazide diuretics, and inhibitors of angiotensin converting enzyme (ACE).[1,2]

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A number of patients with diseases of the cardiovascular system simultaneously take anti-inflammatory drugs due to the presence of concomitant chronic inflammatory diseases such as rheumatism, arthritis, and podagra. However, a

disturbance of vascular tonus during the administration of AC can aggravate the course of inflammation due to hemodynamic disturbances. This issue of practical interest remains unresolved. Considering the above, it seems important to study the effect of AC on the course of the inflammatory process. The development of drugs with anti-inflammatory activity is an urgent task of pharmacology since this typical pathological process forms the basis of the pathogenesis of many human diseases. Despite the large number of drugs using in the treatment of acute and chronic inflammatory diseases, the effectiveness of their pharmacotherapeutic action is poor, since the development of a number of side effects, which pose a serious threat to the life of patients, reduces their value. For this reason, the modern range of anti-inflammatory drugs does not solve the problem of successful therapy of inflammatory diseases and their relapses. The frequency of relapses can reach high numbers after the withdrawal of this group of drugs. According to the requirements of preclinical studies of new drugs, anti-inflammatory properties should be tested in an experiment on models of inflammation induced by various flogogens. The anti-inflammatory activity of calcium antagonists in carrageenan-induced inflammation remains unexplored. This circumstance determined the purpose of this work. Considering the above, it seems important to study the effect of CA on the course of the inflammatory process.

Purpose of the study was an investigation of the effect of amlodipine, diltiazem, and cinnarizine in comparison with diclofenac sodium on the course of carrageenan-induced aseptic arthritis.

MATERIALS AND METHODS

All experiments were carried out on sexually mature male rats with an initial weight of 150–170 g. They kept under standard vivarium conditions with free access to food and water, in a well-ventilated room and day/night light mode, in standard plastic cages, 6 individuals in each, at room temperature 20–24°C. All animals obtained from the vivarium of the Sanitary and Epidemiological Surveillance Department Main medical department under the Administration of the President of the Republic of Uzbekistan. Before starting the experiment, all laboratory animals were carefully examined, weighed, their age, sex, and physical activity were taken into account. All laboratory animals participating in the experiment had a healthy appearance and were active before the beginning of the experiment. The following medicines were used in the experiments: Diclofenac sodium; diltiazem, amlodipine, and cinnarizine. The approval for experiment was taken by the National Ethic Committee of Uzbekistan before beginning of study. The experiments were carried out in accordance with the "Rules for laboratory work using experimental animals," as well as the rules given in the European Convention for the Protection of Vertebrate Animals used for Experimental Research or Other Scientific Purposes (ETS No. 123) Strasbourg, 18.03.1986. The anti-exudative effect of the medicines was studied on a model of acute inflammatory edema of paw. The inflammation was induced by the injection of a 1% aqueous solution of carrageenan in a volume of 0.1 ml under the plantar aponeurosis of the right hind limb of rats.[3] The studied medicines diclofenac sodium at a dose of 10 mg/kg, amlodipine and diltiazem at doses of 20 mg/kg, and cinnarizine at doses of 50 mg/kg were intragastrically administered to experimental animals 1 h before the injection of carrageenan.[4,5] The control group of rats received an equivalent volume of water. Measurement of the paw volume of animals was carried out using a plethysmometer[6] before and 1, 2, 3, and 4 h after the injection of the carrageenan. The values of anti-inflammatory activity (VAA) in% were calculated using the following formula:

$$VAA = [(Vcon - Vexp) / Vcon] \times 100 = \%$$

where Vcon is the average increase of the volume of the paw in the control animals, Vexp is the average increase of the volume of the paw in the experimental animals.[5] It is generally accepted that if the VAA value exceeds 30%, the medicine is considered to have a pronounced anti-inflammatory effect.[7]

Blood was taken from the tail vein for hematological studies at the peak of the exudation process after the injection of carrageenan. Hematological studies were carried out using a hemoanalyzer BC-3000 (MINDRAY, China).

The obtained research results were processed by the method of variation statistics using the standard StatPlus 2009 software package with an assessment of the significance of indicators (M ± m) and differences in the samples under consideration by the Student's *t*-test. The difference was considered significant at a probability level of 95% and more ($P < 0.05$).

RESULTS

The obtained results were given in Tables 1 and 2.

DISCUSSION

The paw volume of healthy animals increased by 59.4% and more than doubled after 3 and 4 h compared to the initial paw volume after one hour from the injection of carrageenan. Along with, the volume of paw remained increased by 28.4% even after 24 h from the moment of carrageenan injection. It is believed that in the 1st h of carrageenan action, inflammation is due to the action of kinins, and in later periods (3 and 4 h later) – prostaglandins.[8] It can be seen in Table 1 that the increase of paw volume under the influence of carrageenan was noticeably low in animals which received

Table 1: The comparative study of the influence of amlodipine, diltiazem, cinnarizine, and diclofenac sodium to the course of aseptic inflammation in carrageenan-induced edema

Groups	Dose, mg/kg	Paw volume, sm^3				
		Initial	After 1 h	After 2 h	After 3 h	After 4 h
Control	-	0.74±0.02	1.18±0.05 0.44±0.04	1.31±0.05 0.57±0.04	1.48±0.04 0.74±0.04	1.42±0.04 0.67±0.04
Diclofenac sodium	10	0.77±0.04	1.02±0.06 0.25±0.06	1.05±0.05 0.28±0.06	1.10±0.06 0.33±0.06	1.04±0.07 0.27±0.06
Amlodipine	20	0.80±0.03	1.09±0.06 0.29±0.05	1.16±0.07 0.37±0.05	1.26±0.05 0.46±0.05	1.21±0.06 0.41±0.04
Diltiazem	20	0.73±0.05	0.99±0.06 0.24±0.03	1.06±0.07 0.31±0.04	1.15±0.06 0.40±0.04	1.11±0.06 0.36±0.04
Cinnarizine	50	0.69±0.04	0.96±0.09 0.27±0.07	1.03±0.10 0.34±0.07	1.12±0.10 0.43±0.07	1.07±0.09 0.37±0.07

In numerator-paw volume; in denominator – difference of edema of rat's paw corresponding term of measuring. *Statistically significant in comparison with initial value (P<0.05)

Table 2: The comparative study of the influence of amlodipine, diltiazem, cinnarizine, and diclofenac sodium to the hematological parameters in carrageenan-induced edema

Groups	Leucocytes, 10^9/l	Absolute lymphocytes count, 10^9/l	Absolute count mix of monocytes, basophiles, eosinophils, 10^9/l	Granulocytes, 10^9/l	Absolute thrombocytes count, 10^9/l	Trombocrit, %
Intact	13.87±1.39	6.20±0.39	2.39±0.20	5.56±0.57	306.21±20.85	0.330±0.023
Control	40.02±3.04* 30.45±3.09	14.25±0.97* 12.67±0.93	11.36±1.06* 10.12±1.13	11.42±1.08* 12.32±0.79	599.54±35.87* 551.21±34.37	0.588±0.032* 0.522±0.041
Diclofenac sodium	18.79±1.84[a] 16.58±1.42	8.33±0.82[a] 7.67±0.61	4.72±0.39*,[a] 4.51±0.28	7.07±0.65[a] 6.89±0.61	367.39±36.45[a] 353.11±36.04	0.381±0.024[a] 0.361±0.031
Amlodipine	21.89±1.80*,[a] 20.31±2.07	9.44±0.74*,[a] 8.75±0.75	5.51±0.56*,[a] 5.14±0.47	7.98±0.56*,[a] 7.25±0.62	423.85±45.40[a] 389.83±39.05	0.408±0.027[a] 0.389±0.025
Diltiazem	20.59±1.32*,[a] 16.79±0.92	8.92±0.76*,[a] 7.46±0.82	5.16±0.42*,[a] 4.59±0.31	7.23±0.66[a] 6.49±0.71	390.01±32.63[a] 358.25±30.63	0.395±0.024[a] 0.374±0.025
Cinnarizine	21.39±1.03*,[a] 19.82±0.73	9.01±0.62*,[a] 7.93±0.71	5.27±0.40*,[a] 5.01±0.33	7.42±0.62[a] 6.78±0.55	400.01±31.08[a] 351.84±26.14	0.401±0.026[a] 0.384±0.027

*Statistically significant in comparison with intact group (P<0.05), [a]Statistically significant in comparison with control group (P<0.05)

a preliminary reference non-steroidal anti-inflammatory medicine – diclofenac sodium compared to the initial volume, the degree of edema was 32.5% after 1 h, 36.4% – after 2 h, 42.8% after 3 h, and 35.1% by the end of the 4th h. At the same time, the volume of paws in rats preventively received a typical representative of the group of non-steroidal anti-inflammatory drugs – diclofenac sodium 1 day after the beginning of the experiment practically did not differ from the values of the initial level. The anti-inflammatory activity of sodium diclofenac in the first phase of carrageenan action was 43.2% and in the second phase was 55.4–60.3%. It can be seen that sodium diclofenac has a more expressed effect on the prostaglandin phase than the kinin phase of inflammation. From the data shown in Table 1, it can be seen that suppression of the intensity of carrageenan-induced aseptic arthritis was also notable under the influence of amlodipine. Hence, in animals which previously received amlodipine, there was an increase of the paw volume 1 h after the injection of carrageenan compared with the initial by 36.2%, and after 2, 3, and 4 h by 45.0%, 57.5%, and 51.5%, respectively, which was distinctly smaller compared to intact ones. The calculation of the anti-inflammatory activity of amlodipine in the indicated hours of observation was 34.1, 36.8, 37.8, and 39.7%, respectively. It can be seen that the medicine has practically the same antiexudative effect regardless of the phases of the flogogenic action of carrageenan. In contrast, a higher anti-inflammatory activity was noted in the third and 4th h of carrageenan action in animals preventively treated with diltiazem. It was more effective in the phase where prostaglandins are the main mediator of inflammation. At the same time, the VAA value of the medicine did not differ significantly from the values of diclofenac sodium. Hence, cinnarizine, like the previous medicine, also had a distinct anti-exudative effect, after 1, 2, 3, and 4 h of the experiment VAA of it were as following: 38.6, 40.3, 41.9, and 44.0%, respectively.

Consequently, the studied medicines have a distinct anti-inflammatory effect. Their pharmacological activity is arranged in a descending order in the following: diclofenac sodium = diltiazem > cinnarizine > amlodipine.

Since the blocking of potential-dependent calcium channels prevents the entry of calcium ions into the vascular smooth muscle cell and exerts a pronounced vasodilating effect by expanding the coronary and peripheral arteries and arterioles, it was assumed that they could enhance the exudation process developing under the influence of flogogenic agents. Moreover, calcium channel blockers significantly accelerate the time of scarring of gastric ulcers and increase their healing process, which indicates that they probably stimulate the formation of prostaglandins (PGE) with a gastroprotective effect.[9] However, the latter cannot emerge in the presence of inflammatory process. It is believed that an increase of calcium in blood is one of the pathogenetic mechanisms contributing to the formation of microcirculatory disorders in which, as is known, progression of inflammatory processes is noted.[9,10] From this point of view, the use of calcium channel blockers in the treatment of inflammatory processes can be considered pathogenetically justified. This is confirmed by the results of this work.

There are studies proving the antioxidant effect of AA.[11] Along with this, an increase in the concentration of calcium in cells promotes the activation of phospholipases leading to the degradation of membrane phospholipids, lysophospholipids, and free fatty acids appear, the function of membranes and their permeability are disrupted.[12] When using modulators of ion channels, the intake of calcium ions decreases, the accumulating capacity of mitochondria restores,[13] the level of calcium ions in the cytosol decreases, and the degree of cell membranes damage decreases accordingly. Based on the above, it can be assumed that the mechanism of antiexudative action of calcium channel blockers might be associated with an increase of the activity of enzymes of the antioxidant system which leads to the suppression of free radical processes, respectively, it decreases damaging of biological membranes while maintaining their physiological permeability.

Effectors of acute inflammation include neutrophils or polymorphonuclear leukocytes (PML), which work not only separately but also in combination with other auxiliary cellular elements in the focus of inflammation (for example, with eosinophils, mast cells, etc.). The role of PML is evidenced by the fact that these cells are the holders of the flogogenic potential (referring a wide range of highly active lysosomal hydrolases, biooxidants, prostaglandins (PG) of group E, leukotrienes), causing the destruction of connective tissue and persistent microcirculation disorders in the inflammation focus. Along with this, neutrophils take place in involving platelets and other cells to the process.[10]

It is shown that distinct changes in the "white blood" were observed in carrageenan inflammation in the results of experimental studies. Thus, in all groups of animals, the introduction of this flogogen caused increase of granulocytes almost two times against the background of pronounced leukocytosis, which was characteristic of an acute inflammatory process. The absolute content of lymphocytes increased by 129.8%, and the absolute content of a mixture of monocytes, basophils, and eosinophils by 375.3%. Against this background, the number of peripheral blood platelets increased by 95.8%, which led to an increase of thrombocytes by 78.2% [Table 2].

Consequently, in the initial phase of carrageenan inflammation, where the kinin system is the main mediator, there was a significant leukocytosis due to a significant increase in the content of lymphocytes in the blood and, especially, granulocytes, which are the main products of inflammatory mediators. It is characteristic that these changes are accompanied by shifts in the platelet content, leading to an increase of clotting processes and the development of thrombosis in the inflammation focus. The similar directional changes of hematological parameters were observed in 3 h after the onset of carrageenan-induced aseptic arthritis. However, the degree of leukocytosis, an increase of the content of granulocytes, especially a mixture of monocytes, basophils, and eosinophils were less more than 2 times in comparison with the 1st h of the previous observation period. At the same time, the number of platelets underwent statistically significant changes, which was high by 80.0%, and thrombocyte was high by 67.3%.

Consequently, the prostaglandin phase of carrageenan-induced inflammation was accompanied by the changes of the hematological parameters of the blood characteristic to the first phase (kinin). Although the degree of these changes was somewhat less expressed.

The obtained results were in accordance with the existing theory of the pathogenesis of inflammation, in which the release of a large number of various mediators and modulators of inflammation during primary and secondary alteration has been proven. It should be noted that in the initial phases of inflammation, the release of vasoactive amine – serotonin is carried out by basophils, mast cells, and platelets, leading to vasodilation, increasing of vascular permeability, and smooth muscle spasm. Further, the release of inflammatory mediators and lysosomal enzymes, neuropeptides is carried out not only by granulocytes, monocytes, macrophages, and platelets but also by cholinergic neurons, C-fibers of afferent neurons, leading not only to vasodilation and increasing of vascular permeability but also tissue destruction, degranulation of mast cells, and stimulation of monocytes and leukocytes, which cause clinical manifestations of inflammation, such as pain and hyperthermia.[10]

Further, we conducted a study to establish the comparative efficacy of sodium diclofenac, amlodipine, diltiazem, and cinnarizine in the correction of hematological disorders in carrageenan-induced inflammation.

It is seen from the data in the table; these substances have a unidirectional effect on the studied indicators. Thus, after 1 h, the level of leukocytosis decreased by 53.0% in animals that received preventatively diclofenac sodium, and by 45.3% in amlodipine, by 48.6% in diltiazem, and by 46.6% in cinnarizine, which was accompanied by a decrease of the total amount of granulocytes, as well as under the influence of sodium diclofenac, amlodipine, diltiazem, and cinnarizine, the absolute content of the mixture of basophils, monocytes, and eosinophils decreased by 58.4%, 51.5%, 54.6%, and 66.0%, respectively. It is noteworthy that these changes were accompanied by a statistically significant decrease not only in the absolute number of platelets but also in thrombocyte in comparison with untreated animals [Table 2].

The results are clearly demonstrated in Table 2 that after 3 h from the beginning of the experiment, the direction of changes of hematological parameters remained the same as in the previous observation period, but their degree was somewhat less expressed. It is noteworthy that the values of the studied hematological parameters of blood were more expressed than the first hours of the experiment under the influence of the investigated medicines.

Consequently, the presented results indicate that diltiazem is not inferior to the classical nonsteroidal anti-inflammatory medicine diclofenac sodium in terms of its effectiveness in correction of alterations of hematological parameters of peripheral blood. This conclusion is confirmed by the results of the study of anti-inflammatory activity of these medicines in carrageenan-induced arthritis model.

Thus, the significant changes of hematological parameters characteristic to acute inflammation are determined in carrageenan-induced aseptic inflammation. It is known that changes of the peripheral hematological parameters of blood reflect the level of compensatory-adaptive reactions of the body. At the same time, the adaptive restructuring of cell functions under the action of the flogogenic agent leads to disorders at the tissue and systemic levels, which cause to the development of the disease. The results of this investigation were showed that CA probably leads to the intensifying of the compensatory-adaptive reactions.

CONCLUSIONS

The anti-inflammatory activity of sodium diclofenac was 43.2% in the kinin phase of carrageenan-induced inflammation, and it was more than 55.0% in the prostaglandin phase. Blockers of potential-dependent calcium channels clearly suppress the exudative phase of aseptic inflammation. The studied medicines are arranged in a descending order by their pharmacological activity in the following row: diclofenac sodium = diltiazem > cinnarizine > amlodipine. Calcium channel blockers and diclofenac sodium equally reduce the level of leukocytosis, restore the quantitative level of lymphocytes, granulocytes, basophils, and eosinophils in aseptic inflammation induced by carrageenan.

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