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Specific Features of Exudative and Proliferative Phase of Inflammation When Using Calcium Channel Blockers

Z. Z. Khakimov[1], A. Kh. Rakhmanov[1,*], N. B. Bekova[2], K. Sh. Shukurlaev[2]

[1]Pharmacology and Toxicology Department, Tashkent Medical Academy, Tashkent, Uzbekistan
[2]Urgench Branch of the Tashkent Medical Academy, Uzbekistan

Abstract The effect of calcium channels blockers (CCB), such as diltiazem, amlodipine and cinnarizine was evaluated to explore the course of aseptic inflammation in sexually mature male rats. CCB in experimental animals show a remodeling effect on the exudative phase of inflammation, providing a distinct non- inferior anti exudative effect in large doses, compare to the classic nonsteroidal anti-inflammatory drug like diclofenac sodium. Interestingly, all investigated drugs suppress not only the exudative but also the proliferative phase of inflammation. Based on the results obtained, we recommended revising the structure of pharmacotherapy in patients taking into account comorbid diseases, the etiology of the inflammatory process of various localization.

Keywords Calcium channels blockers, Anti-inflammatory effect, Inflammation

1. Introduction

Inflammation as a central link in the pathogenesis of many human pathologies is an urgent problem of modern medicine because, despite the introduction of a huge number of drugs of steroid and non-steroidal structures in the treatment of inflammatory diseases, especially chronic ones, there are still unresolved problems. This is largely due to the development of a number of side effects of medications [1,2,3,4], as well as the suppression of prostaglandin synthesis due to blockade of cyclooxygenase activity. In this regard, pharmacologists are faced with the task of creating new effective drugs with a different mechanism of anti-inflammatory action compare to the current ones.

We have previously shown the anti-exudative effect of one of the representatives of calcium channel blockers (CCB) and cinnarizine [5]. However, in-depth studies of other drugs of this group could potentially be used for indications like non-steroidal anti-inflammatory drugs (NSAIDs), because calcium as an intracellular messenger plays an important role in the implementation of the functional activity of cells and its blockade would probably allow suppression of the inflammatory process. Proceeding from this, the purpose of this work was to study a number of CCB medications to investigate the course of aseptic inflammation.

* Corresponding author:
dr.ali.fl@mail.ru (A. Kh. Rakhmanov)
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2. Material and Methods

2.1. Experiments

All experimental studies were conducted on sexually mature male rats with an initial weight of 165-180 g. Animals were kept in standard vivarium conditions with free access to food and water, natural change of light and dark cycles, at room temperature 20-24℃. Animals were obtained from the vivarium of the Sanitary and Epidemiological Department of the Main Medical Directorate under the Administration of the President of the Republic of Uzbekistan.

The following preparations were used in the experiments such as diclofenac sodium (JSC "Sintez", Russia); diltiazem Lannacher (Lannacher Heilmittel GmbH, Austria), amlodipine (RUE Belmedpreparaty, Belarus), cinnarizine (Experimental Plant GNTSLS, Ukraine). Experimental studies 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 for Other Scientific Purposes (ETS No. 123) Strasbourg, 03/18/1986.

Each group of the study contained 6 animals. The anti-exudative effect of the drugs was studied on a model of acute inflammatory edema of the rat paw induced by the introduction of 0.1 ml of 6% dextran solution and 0.1% histamine solution under the plantar aponeurosis of the right hind limb of the animal [6,7]. Diclofenac sodium at a dose of 10 mg/kg was administered into the stomach by metallic cannula to experimental animals 1 hour prior the induction of the inflammation; amlodipine and diltiazem at doses of 5, 10,

and 20 mg/kg, cinnarizine at doses of 10, 25 and 50 mg/kg, respectively. The control group included rats received an equal volume of distilled water. The volume of the right hind paw of the animals was measured using a plethysmometer at the baseline before any manipulations, and 1, 2, 3, and 4 hours after the injection of the flagogen. Based on the data of the average increase in the volume of the paws of animals, obtained as a result of parallel measurements, the values of anti-inflammatory activity (AIA) in % were calculated using the formula:

$$AIA = [(Vk - Vo) / Vk] \times 100 =\%$$

Where Vk is the average increase in the volume of the limb in the control, Vo is the average increase in the volume of the limb in the experiment [5]. If the PVA value exceeds 30%, then, as is commonly believed, the drug has a pronounced anti-inflammatory effect [8].

The investigated drugs were also studied for the proliferative phase of inflammation on the model of "cotton granuloma" [9,10]. This model was created by implanting a sterile cotton swab (10 mg of weight) in rats under the skin of the back between the scapulas. The operation was performed under aseptic conditions using general anesthesia. Injection of the medications into the stomach was carried out once daily before meals, the day of the operation, and every day next seven days in the following doses: Diclofenac sodium 10 mg/kg, amlodipine, and diltiazem in a dose of 20 mg/kg each, and cinnarizine 50 mg/kg. Control animals received distilled water in the comparable volume. 24 hours after the last administration of the drugs (on the eighth day), the animals were sacrificed under general anesthesia, and cotton balls with the granulation tissue formed around them were removed, weighed on an electronic balance (SINKO, Japan, 2014), and dried at 60℃ to constant weight. The degree of the proliferative phase was estimated by the difference between the mass of the dried granuloma and the initial mass of the ball. The exudative reaction was assessed by the difference between the weights of the raw and dried granulomas [11,12].

2.2. Statistical Analysis

The standard StatPlus 2009 software package and the method of variation statistics was used. Continuous data is presented as (M ± m), and the statistical differences between groups was calculated using Student's t-test. The difference was considered significant at a probability level of 95% or more ($p < 0.05$).

3. Results and Discussion

The results of the experiments showed that CCB medications had a definite remodeling effect on the exudative process of inflammation. Thus, the control group of rats after supplantation of dextran the paw volume increases after 1 hour by 161.0%, after 2 hours - by 148.1%, after 3 hours - by 136.4%, and after 4 hours by 123.3% compared to the baseline data. In rats receiving amlodipine at a dose of 5 mg/kg, there were 146.5, 131.5, 116.4, and 102.7% increase, respectively. Under the influence of amlodipine, the increase in the paw volume of rats was less significant compare to controls. At the same time, the calculation of AIA in the indicated study periods was 13.7, 15.8, 19.0, and 21.0%, respectively. As can be seen from the data in Table 1, an increase in the dose of the drug by two and four times led to an increase in the noted effect, especially at a dose of 20 mg/kg.

Consequently, the assumption that vasodilation and a decrease in blood pressure should intensify the exudation process was not confirmed in our experiments. Moreover, with an increase in the dose of the drug, the degree of hypotension should probably increase, however, the exudation process, as can be seen from the data presented, on the contrary, decreases. It was necessary to establish whether this effect was associated with the pharmacological properties of amlodipine particularly, or this is specific to all calcium channel blockers. To explore this, under similar experimental conditions, we conducted a study to examine the effect of another CCB diltiazem. As can be seen from the data in tables 1, the action of diltiazem was similar to amlodipine but slightly more pronounced. If at the peak of exudation the AIA of amlodipine at a dose of 20 mg/kg was 27.4 - 45.3%, then for diltiazem at a similar dose was 34.7 - 46.3%.

The CCB amlodipine, diltiazem has a distinct anti-exudative effect. It is known that these drugs may have general anti-spasmatic effects by lowering the tone of the cerebral vessels, enhancing cerebral circulation. Cinnarizine is one of the drugs that have a relatively selective cerebrovascular effect, as a result of which not only cerebral but also coronary circulation improves [13,14]. It is believed that the drug, along with the fact that it reduces the response to biogenic vasoconstrictor substances (such as angiotensin), also has antihistaminic activity [13].

It is known that histamine is one of the important mediators of inflammation [15], based on this, it can be assumed that cinnarizine may have an anti-exudative effect. The results of experimental studies in animals preemptively receiving cinnarizine, the degree of the exudation process decreased. As can be seen from the data in Table 1, almost identical results were obtained by us in animals that were previously administered with cinnarizine. Thus, in rats previously treated with cinnarizine at a dose of 10 mg/kg, the paw volume increased by 130.2, 115.8, 101.3, and 86.8%, respectively, after 1, 2, 3, and 4 hours from the beginning of the experiment. Under the influence of the cinnarizine at a dose of 25 and 50 mg/kg, the paw volume increased by 125.0 and 92.8% after 1 hour, 108.3, and 80.7% after 2 hours, and by 93.1% after 3 hours, and 69.9% and after 4 hours by 75.0 and 59.0%. The values of anti-inflammatory activity were 37.0 - 44.0% (at a dose of 25 mg / kg) and 37.9 - 48.4% (at a dose of 50 mg / kg), respectively, after 1 - 4 hours of observation.

Table 1. Anti-exudative activity of Diclofenac sodium and Calcium channel blockers in the model of dextran edema in experimental animals

Groups	Dose, mg/kg	Paw volume, см^3				
		initial	1 hour	2 hours	3 hours	4 hours
Control	-	0,77 ± 0,05	2,01 ± 0,07*	1,91 ± 0,07*	1,82 ± 0,06*	1,72 ± 0,06*
Diclofenac	10	0,72 ± 0,02	1,46 ± 0,04*	1,35 ± 0,04*	1,25 ± 0,05*	1,13 ± 0,05*
Amlodipine	5	0,73 ± 0,02	1,80 ± 0,04*	1,69 ± 0,04*	1,58 ± 0,03*	1,48± 0,03*
Amlodipine	10	0,72 ± 0,03	1,70 ± 0,09*	1,58 ± 0,09*	1,47 ± 0,08*	1,36 ± 0,08*
Amlodipine	20	0,76 ± 0,04	1,66 ± 0,06*	1,48 ± 0,06*	1,38 ± 0,03*	1,28 ± 0,03*
Diltiazem	5	0,73 ± 0,04	1,77 ± 0,08*	1,67 ± 0,09*	1,54 ± 0,09*	1,44 ± 0,08*
Diltiazem	10	0,79 ± 0,06	1,68 ± 0,08*	1,60 ± 0,09*	1,50 ± 0,07*	1,42 ± 0,07*
Diltiazem	20	0,74 ± 0,03	1,55 ± 0,07*	1,46 ± 0,06*	1,35 ± 0,06*	1,25 ± 0,05*
Cinnarizine	10	0,76 ± 0,02	1,75 ± 0,05*	1,64 ± 0,06*	1,53 ± 0,05*	1,42 ± 0,04*
Cinnarizine	25	0,72 ± 0,02	1,62 ± 0,09*	1,50 ± 0,09*	1,39 ± 0,09*	1,26 ± 0,08*
Cinnarizine	50	0,83 ± 0,03	1,60 ± 0,06*	1,50 ± 0,07*	1,41 ± 0,07*	1,32 ± 0,06*

Note:*-significant compared to initial volume of paw (P <0.05).

Table 2. Comparative study of the effect of amlodipine, diltiazem, and cinnarizine on the course of aseptic arthritis induced by histamine

Groups	Dose, mg/kg	paw volume, см^3					
		Initial	30 min	60 min	120 min	180 min	240 min
Control	-	0,66 ± 0,05	1,74 ± 0,05*	1,67 ± 0,05*	1,58 ± 0,05*	1,46 ± 0 ,06*	1,38 ± 0,05*
Diclofenac	10	0,63 ± 0,03	1,33 ± 0.08*	1,24 ± 0,08*	1,13 ± 0,07*	1,04 ± 0,04*	0,95 ± 0,05*
Amlodipine	20	0,68 ± 0,02	1,47 ± 0,07*	1,40 ± 0,07*	1,32 ± 0,06*	1,21 ± 0,06*	1,14 ± 0,05*
Diltiazem	20	0,62 ± 0,04	1,36 ± 0,06*	1,28 ± 0,05*	1,20 ± 0,05*	1,11 ± 0,04*	1,02 ± 0,04*
Cinnarizine	50	0,64 ± 0,03	1,36 ± 0,07*	1,28 ± 0,07*	1,21 ± 0,07*	1,11 ± 0,07*	1,03 ± 0,07*

Note:*-significant compared to initial volume of paw (P <0.05).

Consequently, cinnarizine in experimental animals has an anti-flagogenic effect, manifested in a decrease in the exudation process on the effect of dextran.

The classic representative of NSAIDs as diclofenac sodium statistically significantly suppressed the exudative phase of inflammation induced by dextran by 40.3 - 56.8%, respectively, at 1-4 h time points. These data are in agreement with the results of many researchers [6,7,16].

The reason for this effect probably lies in the fact that cinnarizine has an antihistamine effect [13]. In addition, the drug has the properties of a blocker of potential-dependent calcium channels, which leads to the loss of the function of calcium ions acting as an "intermediary" between membrane receptors and intracellular processes [14,16]. In this case, it is necessary to take into account the presence of a certain relationship between cyclic AMP and the kinetics of calcium ions. To establish the mechanisms of the anti-inflammatory activity of cinnarizine, additional pharmacological studies are required.

It is believed that the development of aseptic inflammation induced by dextran is due to the release of histamine and serotonin from mast cells, which are one of the important mediators of inflammation [17]. Based on this, in a separate series of experiments, we investigated the effect of CCB medications and diclofenac sodium on the course of histamine inflammation.

Under the influence of histamine, there is a pronounced increase in exudation processes. Thus, in control rats, histamine led to an increase in paw circumference by 163.6% in 30 minutes after its administration. Subsequently, the effect gradually weakened, however, even by the end of 4 hours of the experiment, the circumference of the paws exceeded the baseline by 2.1 times. In contrast, in rats that were previously injected with amlodipine at a dose of 20 mg/kg, the degree of increase in the paw volume, 30 minutes after the injection of flagogen, was less and amounted to 2.2 times as compared with the initial one. At the same time, the AIA value was 26.8%, 28.7%, 34.4%, 33.7, and 36.1%, respectively, in the studied time periods. In the group of animals previously treated with cinnarizine and diclofenac sodium, the increase in paw volume after administration of histamine was less pronounced than in the group previously treated with amlodipine and diltiazem at doses of 20 mg/kg (Table 2). Cinnarizine at a dose of 50 mg/kg, in terms of its anti-exudative activity, was almost at the level as diclofenac sodium and slightly exceeded those of amlodipine and diltiazem.

Histamine has a multifaceted effect on the human body, in particular, by stimulating H1-receptors located in the vessels, bronchi, and in the stomach, causing an increase in vascular permeability, bronchospasm, a decrease in blood pressure, increased secretion of gastric juice [17,18].

Histamine at the site of inflammation is essentially found simultaneously with the onset of damage. It causes the expansion of the vessels of the microvasculature, increases their permeability, stimulates the endings of pain nerves.

Histamine "triggers" an acute inflammatory response. The appearance of histamine in the inflammation is closely related to the degranulation of mast cells, because of the initiated synthesis of new mediators from the lipids of the activated mast cells and basophils membranes, such as proteases, proteoglycans, eosinophil chemotaxis factors, kinins, complements, eicosanoids, leukotrienes (activation factor T) and others [17].

It is known that inflammation is a protective reaction of the body against various factors, which manifests itself at the site of tissue or organ damage by increasing vascular permeability, changes in blood circulation and cell proliferation, which subsequently leads to multiple organ damage, a decrease in the quality of life and a deterioration in the prognosis of the disease [19]. The suppression of the exudative phase of inflammation will be accompanied by a decrease in the proliferation process in the inflammation since this direction of changes is characteristic of the action of many anti-inflammatory drugs. This circumstance required a study of the effect of CCB on the proliferative phase of inflammation.

Our study showed that the classic representative of NSAIDs diclofenac sodium reduces the formation of granulomas in comparison with the control by 34.0%. As can be seen from the data in Table 3, almost the same degree of inhibition of proliferation was observed in rats receiving cinnarizine and diltiazem, and to a somewhat less pronounced degree in rats receiving amlodipine. All investigated drugs suppress not only the proliferative but also the exudative phase of inflammation. The latter once again confirms the validity of the conclusions drawn on the basis of the results of the previous series of experiments. Therefore, CCB is characterized by the suppression of the inflammatory process. The major explanation for this effect is probably a decrease in the formation and release of inflammatory mediators due to the loss of the function of calcium ions, which are known to act as an “intermediary” elements between membrane receptors and intracellular structures [14,17]. In this case, it is necessary to take into account the presence of a certain relationship between cAMP and the calcium ions. CCB medications probably also inhibit the activity of enzymes involved in the synthesis of prostaglandins and cytokines that have an inflammatory effect. To establish the final mechanisms of the anti-inflammatory activity of CCB medications, additional pharmacological studies are needed.

Table 3. Anti-exudative and anti-proliferative activity of diclofenac sodium and calcium channel blockers in rats in the inflammatory process

Groups	Dose, mg/kg	Wet mass, mg	Dry weight, mg	Difference, mg
Control	-	336,33 ± 12,27	76,01 ± 4,49	260,33 ± 23,53
Diclofenac	10	193,17 ± 15,28*	50,16 ± 5,01*	143,02 ± 10,32*
Amlodipine	20	252,17 ± 11,70*	57,67 ± 3,52*	194,83 ± 8,25*
Diltiazem	20	223,67 ± 8,71*	52,50± 4,05*	171,17 ± 5,95*
Cinnarizine	50	211,33 ± 12,11*	51,83 ± 5,13*	159,5 ± 7,49*

Note: * - significant compared to control group (P <0.05).

4. Conclusions

1. Calcium channel blockers such as cinnarizine, diltiazem, and amlodipine in experimental animals have a modeling effect on the exudative phase of inflammation, providing a distinct anti-exudative effect in higher doses, compare to the classic nonsteroidal anti-inflammatory medication like diclofenac sodium.

2. The investigated drugs suppress not only the exudative but also the proliferative phase of inflammation.

3. The mechanism of the anti-inflammatory action of calcium channel blockers is probably associated with a decrease in the release of inflammatory mediators in connection with the loss of the function of calcium ions acting as a secondary messenger.

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