Anti-Inflammatory Effects of L-Type Calcium Channel Blockers

L-TİPİ KALSİYUM KANAL BLOKERLERİNİN ANTİ-İNFLAMATUAR ETKİLERİ

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Abstract

Objective: Calcium ion has an important role in the synthesis and release of chemical mediators during inflammation. The aim of this study was to examine the anti-inflammatory effects of L-type calcium channel blockers on acute and chronic inflammation models in rats and also to examine the effects of nicardipine on capillary vascular permeability in rabbits.

Material and Methods: Effects on acute phase of inflammation of verapamil, diltiazem and nicardipine (10 mg/kg doses) were compared with diclofenac sodium (25 mg/kg) in histamine-induced paw edema model. After measuring the right hind paw volumes of rats, drugs were injected intraperitoneally 30 minutes later. Paw edema was induced by 0.1 mL subplantar histamine (0.1%) injection to the same paw. Subsequent paw volumes were measured at 30 minutes intervals. Effects of the drugs on the chronic phase were tested with cotton pellet granuloma method in rats, and effect of nicardipine on capillary vascular permeability was examined with hyaluronidase test in rabbits. Results were compared with control groups.

Results: In acute inflammation model, after the histamine injections, maximal paw edema was observed at 30 min. While verapamil and diltiazem did not have an anti-inflammatory effect, nicardipine and diclofenac sodium showed significant anti-inflammatory activities of 63.77% (p= 0.000) and 42.93% (p= 0.002), respectively. While verapamil and diltiazem had similar effects to those of the control group and to each other (p> 0.05), nicardipine had the most potent activity (p< 0.05). Also, nicardipine (3 mg/kg) significantly reduced hyaluronidase-induced capillary permeability (p= 0.000). Calcium channel blockers did not show anti-proliferative effects oi the chronic phase of inflammation,

Conclusion: Since nicardipine, a calcium channel blocker significantly inhibited histamine-induced acute inflammation and the dissemination area of hyaluronidase, its anti-inflammatory effect may be due to its higher vaso-selectivity on peripheral vascular smooth muscles and on preventing the increase of vascular permeability.

Key Words: Calcium channel blockers; histamine; inflammation; nicardipine

Özet

Amaç: Kalsiyum iyonu inflamasyon esnasında kimyasal mediyatörlerin sentez ve salıverilmesinde önemli rol oynar. Çalışmamızın amacı L-tipi kalsiyum kanal blokörlerinin sıçanlarda oluşturulan akut ve kronik inflamasyon modellerinde anti-inflamatuar etkilerini araştırmak ve nikardipinin tavşanlarda kapiller vasküler permeabilite üzerine etkisini değerlendirmektir.

Gereç ve Yöntemler: Sıçanlarda histamin ile oluşturulan pençe ödemi testinde, verapamil, diltiazem ve nikardipinin (10 mg/kg dozlarda) inflamasyonun akut fazına etkileri, diklofenak sodyum (25 mg/kg) ile karşılaştırıldı. Hayvanların sağ arka ayak hacimleri ölçüldü, intraperitoneal ilaçlar enjekte edildi. 30 dk. sonra, aynı ayaklarda %0.1'lik histamin solüsyonu ile inflamasyon oluşturuldu. Histamin enjeksiyonlarını takiben, 30 dk. aralıklarla hayvanların pençe ödemleri ölçüldü. Kalsiyum kanal blokerlerinin kronik faza etkileri Cotton-Pellet granüloma methoduyla ile ve nikardipinin kapiller vasküler permeabilite üzerine etkisi tavşanlarda hiyalurinidaz testi ile değerlendirildi. Sonuçlar kontrol grupları ile karşılaştırıldı.

Bulgular: Akut inflamasyon modelinde, histamin enjeksiyonundan sonra maksimum pençe ödemi 30. dk.da gözlendi. Verapamil ve diltiazem anti-inflamatuar etki yapmazken, nikardipin ve diklofenak sırasıyla %63.77 (p= 0.000) ve %42.93 (p= 0.002)'lük anti-inflamatuar etki gösterdiler. Diğer ölçümlerde de verapamil ve diltiazem kontrol grubuna ve birbirine benzer etkiler gösterirken (p> 0.05), nikardipin en güçlü anti-inflamatuar etkiyi sergiledi (p< 0.05). Ayrıca, nikardipin hiyaluronidaz ile oluşturulan kapiller permeabiliteyi de azalttı (p= 0.000). İnflamasyonun kronik fazında kalsiyum kanal blokörleri antiproliferatif etki göstermediler.

Sonuç: Kalsiyum kanal blokörlerinden nikardipin histamine ile oluşturulan akut inflamasyonu ve hiyolurinidazın yayılma alanını belirgin olarak inhibe ettiğinden dolayı göstermiş olduğu anti-inflamatuar etkisi periferal vasküler düz kaslarda daha vazoselektif olmasına ve vasküler permeabilite artışını önlemesine bağlı olabilir.

Anahtar Kelimeler: Kalsiyum kanal blokerleri; histamin; inflamasyon; nikardipin

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Calcium channel blockers, especially used in the treatment of various cardiovascular system diseases, inhibit the influx of extracellular calcium through the L-type channel, resulting in relaxation of vascular smooth muscle and reduction in vascular resistance.[1,2]

Inflammation is involved in the pathogenesis of various diseases, and the special components of inflammation are hemodynamic changes, polymorphonuclear leukocyte infiltration and secretion of inflammatory mediators.[3]

Anti-inflammatory mechanisms of steroidal and/or non-steroidal anti-inflammatory drugs used in the therapy of inflammatory diseases are dependent on inhibiting of the synthesis of chemical mediators during inflammation.[4-6] Calcium ion plays an important role in the synthesis and release of chemical mediators of inflammation.[1,7,8] While increased calcium may potentiate nociception or inflammatory events, decreased calcium may reduce such events.[9,10] Intra-dermal injection of calcium results with acute inflammation.[11] Again, calcium interacts with various analgesic and anti-inflammatory drugs, which partly inhibit calcium influx via calcium channels; therefore, intracellular calcium level is decreased by these drugs.[11,12]

Inflammatory process has two phases: Acute and chronic. Acute inflammation is characterized by fever, pain, and edema, whereas the characteristic of chronic inflammation is cellular proliferation. Complement system, fibrinolytic system and hyaluronidase enzyme in plasma are activated during inflammation.[3] Hyaluronidase activity in blood is increased during inflammation and the reduction of the inflammation parallels a decreasing in hyaluronidase activity.[13] Acute inflammation models are induced by histamine, carrageenan, serotonin, formaline, dextran, bradykinin, and prostaglandin. Models of chronic inflammation, which is provoked by subcutaneous (*sc*) implantation of foreign bodies, are used to investigate the effects of drugs on a chronic phase of inflammation.[14]

In this study, we aimed to evaluate the acute and chronic anti-inflammatory effects of the different L-type calcium channel blocker drugs (verapamil, diltiazem and nicardipine) and to compare them with diclofenac sodium and each other. In addition, we evaluated the effect of nicardipine in the hyaluronidase-induced capillary permeability test in rabbits.

Material and Methods

Animals: Male Sprague-Dawley rats (175-200 gr) and rabbits (3.5-4 kg), which were obtained from the Atatürk University Pharmacology Laboratory and were nourished under normal conditions, were used. Each experimental group consisted of six animals. The study was performed according to the international, national and institutional rules considering animal experiments rights.

Drugs: Verapamil (Knoll- Turkey), diltiazem (Mustafa Nevzat AŞ-Turkey), nicardipine (Sigma-USA), diclofenac sodium (Fako AŞ-Turkey) and histamine (Sigma-USA) were dissolved in distilled water. Verapamil, diltiazem, nicardipine and diclofenac sodium were administered 1 mL intraperitonally (ip). Hyaluronidase was obtained from Sigma-USA, and dissolved in NaCl 0.9% solution.

Histamine-induced paw edema:[15] In preliminary experiments, ip administrations of verapamil, diltiazem and nicardipine did not produce any detectable edema. After measuring normal paw volumes of the animals using a plethysmometer (model 7140; Ugo Basile, Milan, Italy), verapamil (10 mg/kg), diltiazem (10 mg/kg), nicardipine (10 mg/kg), diclofenac sodium (25 mg/kg) for the study groups and distilled water for the control group were administrated by ip injection 30 minutes later. Paw edema was induced in rats by subplantar injection of 0.1 mL histamine (0.1%) to the right hind paw. Subsequent volume readings for the same paw were carried out at 30- minute intervals. Results were measured as percentage differences versus initial volumes. The ratio of anti-inflammatory activity of the drugs was calculated by the following equation:

Anti-inflammatory activity (AIA-%)= (1-*D/C*) x 100.

D represents the percentage difference of paw volume after drug administration and *C* represents the percentage difference of paw volume in the control group.

Cotton-pellet granuloma test:[14] Verapamil (10 mg/kg), diltiazem (10 mg/kg), nicardipine (10 mg/kg), diclofenac sodium (25 mg/kg) and distilled water (1 mL) were administrated by ip injec-

tion 20 minutes after autoclaved sterile pellets of cotton, weighing 10 ± 1 mg each, were aseptically implanted in the inter-scapular distance under the skin on the previously shaved back of the rats which were anesthetized with ip injection of thiopental sodium 25 mg/kg. The same doses of the drugs were given once a day for a period of seven days. Control group was nourished with the same volume of water. On day eight, the rats were killed by decapitation and the pellets surrounded with granuloma tissue were dissected out carefully and dried at 70°C. The mean weight of the granuloma tissue formed around each pellet was recorded. The pellets were weighed both moist and dry. The weight of the pellets extracted from study rats that received drugs were compared to the weight of pellets removed from the control group and from the diclofenac sodium administered rats.

Hyaluronidase-induced capillary permeability test:[14] In this section of the experiment, the effects of nicardipine (3 mg/kg) and diclofenac sodium (5 mg/kg) on hyaluronidase-induced capillary permeability were investigated. The rabbits (n= 18) were divided into three equal groups and their abdominal hair were shaved. The first group received nicardipine, while the second group received diclofenac sodium through oral catheter. The third group (control) received only the same amount of vehicle (NaCl, 0.9%). Hyaluronidase (128 units) was dissolved in 1 mL isotonic NaCl. Trypan blue (0.8 mL of 0.75%) was added to this hyaluronidase solution (0.5 mL). The last mixture (0.1 mL) was injected subcutaneously in the abdominal region after 1 h of each oral drug administration. The appearance of the blue area was measured after 1, 5 and 30 min of injection as mm^2. The size of the blue area corresponds the activity of the hyaluronidase enzyme and capillary permeability, i.e. the smaller the appearance of the blue area, the lower the activity of the hyaluronidase enzyme and capillary permeability. The results obtained were compared with those of the control group.

Statistical Methods

Values are presented as mean ± SEM. Statistical analysis was performed by ANOVA and post-hoc LSD test. The significance level was accepted as $p< 0.05$.

Results

Effects of Ca^{2+} channel blockers on histamine-induced paw edema:

In preliminary experiments, Ca^{2+} channel blockers used in our study did not produce any detectable edema in the non-treated paw. After histamine injection, edema of the rat paws developed within 30 min (35.03 ± 3.37%), decreased as in the following measurements. Maximal edema was observed at 30 min (Table 1). At 30 min, in

Table 1. The effect of calcium channel blockers on paw volume after histamine injections. Data are expressed as mean ± SEM (%), for n=6 rats.

Drugs	The Changes of Paw Volume at 30 Min Intervals After Histamine Injections (%)											
	30 min	p	60 min	p	90 min	p	120 min	p	150 min	p	180 min	p
Control	35.03 ± 3.3	-	30.05 ± 3.15	-	27.07 ± 5.86	-	22.63 ± 4.79	-	19.44 ± 5.54	-	17.63 ± 4.53	-
	+++		++		+++		+		*ns*		*ns*	
Verapamil	34.15 ± 3.54	*NS*	28.13 ± 5.05	*NS*	26.51 ± 3.69	*NS*	22.43 ± 5.30	*NS*	19.43 ± 5.51	*NS*	15.81 ± 6.65	*NS*
	+++		+		++		+		*ns*		*ns*	
Diltiazem	33.39 ± 3.23	*NS*	25.77 ± 3.58	*NS*	22.01 ± 1.68	*NS*	20.10 ± 1.36	*NS*	19.23 ± 2.34	*NS*	14.42 ± 2.40	*NS*
	+++		*ns*		+		*ns*		*ns*		*ns*	
Nicardipine	12.69 ± 2.31	**	11.55 ± 2.31	***	10.92 ± 2.12	***	5.77 ± 2.62	**	6.84 ± 2.06	*	2.00 ± 0.81	**
	ns	*	*ns*		*ns*		*ns*	*	*ns*		*ns*	
Diklofenac	19.99 ± 2.40	**	16.26 ± 1.99	**	11.50 ± 1.94	***	9.91 ± 2.30	*	10.06 ± 1.98	*NS*	10.64 ± 2.69	*NS*
	-	*	-		-		-		-		-	

NS not significant, * p< 0.05, ** p< 0.01, *** p< 0.005 as compared to control group,
ns not significant, + p< 0.05, ++p< 0.01, +++p< 0.005 as compared to diclofenac sodium group (*post-hoc LSD test*).

comparison to histamine produced edema, while verapamil and diltiazem did not show an anti-inflammatory effect (p> 0.05), nicardipine and diclofenac sodium had anti-inflammatory effects of 63.77% (p= 0.000) and 42.93% (p= 0.002), respectively (Figure 1). For each measurement in the study, while verapamil and diltiazem presented statistically comparable effects to those of the control group and each other (p> 0.05), nicardipine showed the most potent activity (p< 0.05) and had the similar effects to those of the diclofenac sodium group (Table 1, Figure 1).

Effects of Ca^{2+} channel blockers on cotton-pellets granuloma test:

The anti-proliferative effect was calculated on the basis of dry weight pellets, and the inhibition rates of the inflammation with verapamil, diltiazem, nicardipine and diclofenac sodium were established as 0% (p> 0.05), 11.6% (p> 0.05), 9.3% (p> 0.05) and 27.9% (p< 0.05), respectively. According to these results, the anti-proliferative effects of Ca^{2+} channel blockers versus the control group were not statistically significant.

Effects of nicardipine on hyaluronidase-induced capillary permeability test:

The dissemination areas of trypan blue, which was administrated with hyaluronidase, were 155.00 ± 6.19, 316.00 ± 11.06 and 510.80 ± 17.06 mm^2 in the control group, 149.00 ± 7.90 (p> 0.05), 184.33 ± 4.75 (p= 0.000) and 300.00 ± 6.58 (p= 0.000) mm^2 in the nicardipine group, and 87.00 ± 2.84 (p= 0.000), 117.33 ± 5.55 (p= 0.000) and 119.00 ± 5.07

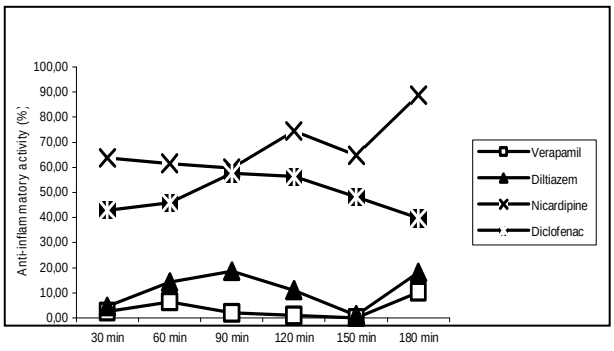

Figure 1. Anti-inflammatory activities (%) of verapamil (10 mg/kg), diltiazem (10 mg/kg), nicardipine (10 mg/kg) and diclofenac sodium (25 mg/kg) on histamine-induced paw edema.

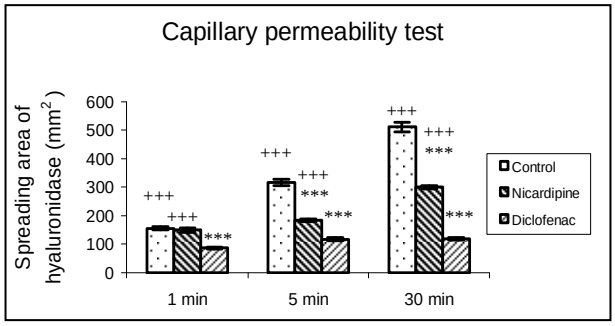

Figure 2. Effects of nicardipine (3 mg/kg) and diclofenac sodium (5 mg/kg) on hyaluronidase-induced capillary vascular permeability in rabbits (Mean ± SEM).
*** p= 0.000 as compared to control group,
+++ p= 0.000 as compared to diclofenac sodium group (*post-hoc LSD test*).

(p= 0.000) mm^2 in the diclofenac group after 1, 5, and 30 min, respectively (Figure 2).

Discussion

In the present study, the anti-inflammatory activities of verapamil, diltiazem and nicardipine were examined in acute (histamine-induced inflammation) and chronic inflammation (cotton-pellet granuloma test) models in the rat. Although nicardipine showed the most potent activity in the acute inflammation model, none of the drugs presented an anti-proliferative effect in the cotton-pellet granuloma test. Later, in hyaluronidase-induced capillary permeability test in the rabbits, nicardipine diminished the hyaluronidase-induced capillary vascular permeability.

Although calcium channel blockers are usually preferred in the treatment of cardiovascular diseases, calcium ion takes part in a plenty of functions in the body. Calcium ion plays a critical role in the formation and secretion of a wide variety of chemical mediators, and calcium channels are targets for a variety of neurotransmitters, neuromodulators and drugs.[16] In inflammation, arachidonic acid metabolites are the most important mediators. In the cell, calcium ion increases the lipooxygenase products by activating 5-lipooxygenase enzyme and eicosanoid synthesis by activating cytosolic phospholipase A_2.[17,18] It is well established that arachidonic acid is metabolized both by cyclooxygenases and lipooxygenases.[19] Also, there is evi-

dence that calcium channel blockers inhibit the lipooxygenase pathway.[20]

Kouoh et al reported that elastase and superoxide anion radicals were inhibited by nicardipine and that this inhibition was dependent on the inhibitor effect on the mobilization of cytosolic calcium and on activation of protein kinase C and suggested that this drug might be useful as an anti-inflammatory drug.[21]

There are a number of studies suggesting anti-inflammatory activity of calcium channel blockers. In the study of Bilici et al, T-type calcium channel blocker (mibefradil) showed a protective effect on the histamine-induced paw inflammation in the rat.[15] Gurdal et al induced paw inflammation by formaline in rat, and nitrendipine and nicardipine (80%), verapamil and diltiazem (30-50%) inhibited the inflammation.[11] In the study of Aditya et al, verapamil, diltiazem and nifedipine blocked the carragenine-induced rat paw inflammation, and nifedipine was more effective than other calcium channel blockers.[22] In both studies, dihydropiridine-derived calcium channel blockers proved to be more effective than diltiazem and verapamil. In our study, the edema was produced with histamine and nicardipine showed a potent anti-inflammatory effect similar to that of diclofenac sodium, but verapamil and diltiazem did not.

L-type calcium channel blockers include three discrete chemical types: the phenylalkylamines (verapamil), the benzothiazepine (diltiazem), and the 1,4-dihydropyridines (nicardipine). Although chemically distinct, the net pharmacologic profiles of verapamil and diltiazem are much closer to one another than either is to the dihydropyridines.[2] In our study, verapamil and diltiazem presented similar effects. The activity of calcium channel blockers in a particular tissue may be influenced by the location of the receptor site and the frequency of channel activity.[2] The verapamil and diltiazem binding sites are located internally, deep within the channel. The 1,4-dihydropyridine receptor is the most accessible, located on the surface of the channel. Although the dihydropyridines act preferentially on peripheral vascular smooth muscle, verapamil and diltiazem are less specific for that region.[12] The chief hemodynamic feature of calcium channel blockers is a reduction in vascular resistance and an improvement in blood flow of the coronary and peripheral arteries.

In the inflammation process, increase in vascular permeability as well as inflammatory mediators have an important role. In this study, acute inflammation was induced by histamine, which is one of the major inflammatory mediators and its major component is the increase of the vascular permeability.[23,24] Another well established characteristic of hyaluronidase is its enhancing effect on vascular permeability as well as causing an increase in tissue permeability by hydrolyzing hyaluronic acid, and non-steroidal anti-inflammatory drugs are known to diminish hyaluronidase-induced capillary permeability.[14] In our study, nicardipine had the most potent anti-inflammatory effect in the histamine-induced inflammation model and this drug significantly reduced hyaluronidase-induced capillary vascular permeability. In the light of these results, we may speculate that the anti-inflammatory effect of nicardipine may be dependent on preventing the enhancement of vascular permeability, since nicardipine is more vasoselective than verapamil and diltiazem on peripheral vascular smooth muscle.

In this study, chronic inflammation was evaluated with cotton-pellet granuloma test. None of the calcium channel blockers displayed an anti-proliferative effect, while diclofenac sodium did. In the literature, a number of reports suggest an anti-proliferative effects of calcium channel blockers.[22,25-27] On the other hand, other reports indicate the induction of gingival fibroblast proliferation with the prolonged use of such drugs.[28] Inflammation has acute and chronic components. Chronic inflammation develops after acute inflammation with the development of proliferative cells. These cells either disseminate or a granuloma is formed. Prevention of the collagen fiber formation and suppression of mucopolysaccharids are indicators of the anti-proliferative effect of anti-inflammatory agents.[29] Monocyte infiltration and fibroblast proliferation is more prominent than neutrophil infiltration and exudation during chronic inflamma-

tion.[30] Calcium ion acts as an important messenger and effector in the attachment and proliferation of fibroblasts. Calcium, when coupled with calmodulin inside the cell, stimulates various enzymes necessary for fibroblast proliferation and function.[25] Calcium also plays a vital role in platelet activation, and activated platelets release several factors, most notably platelet-derived growth factor, which stimulate fibroblast proliferation.[25] Inhibition of protein kinase C may be a mechanism by which calcium channel blockers suppress fibroblast proliferation.[26] Kang et al reported that following glaucoma filtration surgery, these drugs had inhibited fibroblast attachment and proliferation.[25] In the study of Aditya et al, while nifedipine showed an anti-proliferative effect, verapamil and diltiazem did not.[22] Suleyman et al reported that nicardipine had an anti-proliferative activity in the cotton-pellet granuloma test.[27] In fact, although we had expected an anti-proliferative activity particularly for nicardipine in our study, we did not observe such an effect. Reports suggest that prolonged treatment with calcium channel blockers induces gingival hyperplasia in patients with gingival inflammation and dental plaque.[28] Matsumoto et al indicated that gingival fibroblasts obtained from patients reactive to nifedipine and nicardipine medications had a better cell proliferation rate, DNA synthesis and collagen synthesis with nifedipine or nicardipine treatment compared with non-drug-treated controls.[31] Reports indicate that the interaction between calcium channel blockers and gingival fibroplasts is calcium-dependent and that these drugs affect intracellular calcium metabolism or transport in some patients and stimulate gingival fibroblasts resulting in hyperplasia of extracellular matrix components, such as the accumulation of glycosaminoglycans leading to gingival hyperplasia.[32] In our study, the anti-proliferative effect was studied on the back skin of rats and such an effect was not observed; this may be dependent on individual differences and/or reactivity to these drugs.

As a result, in our study, although the chronic anti-inflammatory effects of calcium channel blockers were not significant, nicardipine had a protective effect in the histamine-induced inflammation model, and it reduced the hyaluronidase-induced capillary permeability. Perhaps, nicardipine may be useful to prevent the inflammatory process in the future.

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