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Effects of Calcium Channel Blockers On Hyaluronidase-induced Capillary Vascular Permeability

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Inflammation and increased capillary permeability is a significant aspect of the pathogenesis of many diseases including atherosclerosis. L-type calcium channel blockers (CCB) are commonly used as cardiovascular drugs. Amlodipine, lacidipine, and nicardipine were evaluated for anti-inflammatory activity on the paw oedema produced by carrageenan. The effect of these drugs was compared with the activity of indomethacin. Their effects on vascular permeability were also tested by hyaluronidase-induced capillary permeability. In our animal experiments, amlodipine decreased the carrageenan-induced paw oedema at doses of 1, 3, and 6 mg kg^{-1} by 27.3%, 43.7%, and 67.3% four hour after carrageenan administration; the same doses of lacidipine and nicardipine decreased paw oedema by 37.1%, 55.6%, 76.4%, 11.2%, 31.0%, 91%; and indomethacin decreased oedema by 38.2% at a dose of 6 mg kg^{-1}. Lacidipine significantly inhibited the hyaluronidase-induced increase in capillary permeability at doses of 1, 3, and 6 mg kg^{-1} compared with the control group. However, amlodipine and nicardipine significantly inhibited the hyaluronidase-induced increase in capillary permeability at 3 and 6 mg kg^{-1} doses. A 6 mg kg^{-1} dose of indomethacin significantly decreased the capillary permeability which was increased by hyaluronidase. These results suggest that CCBs can be efficient anti-inflammatories, and can also significantly decrease capillary permeability.

Key words: Amlodipine, Capillary Permeability, Hyaluronidase Enzyme, Lacidipine, Nicardipine

INTRODUCTION

Several types of calcium antagonists (CA) (verapamil, diltiazem, nifedipine and related drugs) may be used in hypertensive disease. In practice, the dihydropyridines (amlodipine, lacidipine and related drugs) are the calcium antagonists used most frequently as antihypertensives, and are called L-type calcium channel blockers (CCB) (Van Zwieten, 1998). As well as lowering blood pressure CAs may have been other, theoretically beneficial effects: regression of left ventricular and vascular hypertrophy, renal protection, weak natriuretic activity, weak antiplatelet activity, as well as anti-ischemic, anti-inflammatory and antiatherogenic activity (Janssen *et al*., 2001). In recent years, several new dihydropyridine CAs have been introduced. These newly synthesized compounds, such as amlodipine, lacidipine, and lercanidipine, have many advantages. These can be summarized as: vasoselectivity, an improved kinetic profile, resulting in a slow onset and long duration of action, reduction of peripheral resistance so little or no cardio depressant activity, fewer side-effects such as reflex tachycardia and headache, related to the slow onset of the antihypertensive action (Abernethy and Schwartz, 1999; Grossman and Meserli, 2004; Oates and Brown, 2006).

Many researchers have explained that amlodipine and lacidipine, the third generation of CCBs, have direct vasculoprotective effects on cells in the arterial wall (Pitt *et al*., 2000). This effect includes amelioration of endothelial dysfunction, and decreases in oxidative stress and inflammation (Chen *et al*., 1997; Cominacini *et al*., 1999).

As has been noted, CCBs show their anti-inflammatory effect by inhibiting the synthesis and release of inflammation mediators. In recent years, researchers have shown that amlodipine inhibits the production of pro-inflammatory cytokines in vivo and in vitro, such as TNF-α, IL1β, and inducible nitric oxide synthase (iNOS) (Mohler *et al*., 1997).

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CCBs inhibited carrageenan and formalin inflamed paw oedema in a dose dependent manner (Aditya *et al*., 1997; Gurdal *et al*., 1997; Kouoh *et al*., 2006; Suleyman *et al*., 2006).

Dihydropyridine derivative CCBs (nifedipine, nisoldipine, nicardipine) are known to decrease the production of inflammation mediators such as prostaglandin (PG), superoxide anion (O^{-2}) and elastase (Kouoh *et al*., 2006). Verapamil possesses an anti-inflammatory effect *via* inhibition of cytokines and nitric oxide (NO) which are known as inflammation mediators (Martinez *et al*., 1999; De Souza *et al*., 2004). CCBs also inhibit both the synthesis of leukotriene and the release of lysosomal enzymes (Elferink, 1982; Chand *et al*., 1985).

The initial molecular and cellular events in atherogenesis are induced by endothelial dysfunction, resulting in decreased NO production, increased cyclooxygenase activity and inflammation (Fuster *et al*., 1992; Libby, 1995). This effect of CCBs is related to the inflammatory response, which is known to have three phases, each mediated by a different mechanism: 1. The acute phase, which shows a classic inflammatory response including calor, dolor, rubor, and tumor, characterized by transient local vasodilatation and increased capillary permeability; 2. The sub-acute phase appears by infiltration of leukocytes; In addition, 3. The chronic phase (proliferative phase) (Maslinska and Gajewski, 1998). The activity of the hyaluronidase enzyme increases during the inflammation and decreases as the inflammation reduces (Procida *et al*., 1971; Houck and Chang, 1979). The role of the hyaluronidase enzyme is also termed the “spreading factor”, as the increase of vascular permeability is known (Houck and Chang, 1979). However, there is no study in the literature on the effects of CCB on the activity of the hyaluronidase enzyme, which has an important role in the acute phase of inflammation, and also in the treatment of inflammation and atherogenesis.

Our study aimed to investigate the effects of amlodipine, lacidipine, and nicardipine on capillary permeability in the hyaluronidase test, and to determine the role of the hyaluronidase enzyme in the action of the anti-inflammatory mechanisms of these drugs. We also investigated the efficiency of CCBs as anti-inflammatories, and showed significant parallel action in decreased capillary permeability.

MATERIALS AND METHODS

Animals

Animals were housed in facilities accredited by the International Guidelines and our studies were approved by, and conducted in accordance with, the Institutional Animal Care and Use Committee. In this study, 66 adult male Wistar albino rats weighing 180-200 g and 85 albino rabbits weighing 3.5-4 kg were taken from Ataturk University Experimental Animal Laboratory of Medicinal and Experimental Application and Research Center to be used in this study. The animals were housed in groups of five or six per cage for at least 5 days under controlled conditions of constant temperature/humidity and exposed to a 12-h light/dark cycle.

Chemicals

Amlodipine (Norvasc 5 mg tablet) supplied by Pfizer Turkey, lacidipine (Lacipil 4 mg tablet) purchased from GlaxoSmithKline, nicardipine (pure substance) purchased from Sandoz Pharma Turkey, and indomethacin (Endosetin capsule) purchased from Nobel Turkey were used in the study. Carrageenan and trypan blue stain was obtained from Sigma (Germany). Hyaluronidase was obtained from Kiyevskoye predpriyatiye po proizvodstvu Bakteriynih preparatov (Kiev, Ukraine). Hyaluronidase (hyaluronoglucosaminidase, EC 3.2.1.35) was obtained from bovine testicles; its specific activity is 0.64 U/mg.

Carrageenan-induced inflammation model in intact rats

The anti-inflammatory activities of amlodipine, lacidipine, nicardipine and indomethacin were evaluated against an experimental model of acute inflammation: paw oedema in rats, using carrageenan (Winter *et al*., 1962). This model is commonly used to screen conventional non-steroidal anti-inflammatory drugs and involves the activation of the arachidonic acid cascade, giving rise to the formation of the principal mediators of inflammation (PGs and thromboxanes). Rats were divided into 11 groups: group 1 (n = 6) consisted of untreated controls [0.9% NaCl 2 mL per oral (p.o.)]. Groups 2, 3, and 4 (n =6, each group) were treated with amlodipine (1, 3, and 6 mg kg^{-1} p.o. using gavages, respectively). Groups 5, 6, 7 (n =6, each group) were treated with lacidipine (1, 3, and 6 mg kg^{-1} p.o. using gavages, respectively). Groups 8, 9, 10 (n =6, each group) were treated with nicardipine (1, 3, and 6 mg kg^{-1} p.o. using gavages, respectively). Group 11 was treated with indomethacin (6 mg kg^{-1} p.o. using gavages). All drugs were dissolved in normal saline (2 mL 0.9% NaCl). Drugs were administered 1 h prior to injection of 0.1 mL of 1% freshly prepared suspension of carrageenan in normal saline in the right hind paw sub-plantar of each rat. The paw volume was measured initially and then at 1, 2, 3, 4, and 5 h after the carrageenan injection, using a plethysmometer. The anti-inflammatory effect of these drugs was calculated using this equation: anti-inflammatory activity (%) = $(1\text{-}D/C) \times 100$, where D represents the percentage difference in paw volume after the administration of drugs to the rats, and C represents the percentage difference of volume in the control groups.

Hyaluronidase test

In this series of experiments, the effects of amlodipine, lacidipine, nicardipine, and indomethacin on hyaluronidase activity was investigated with the hyaluronidase-induced capillary permeability test (Suleyman *et al*., 2007); 55 albino rabbits were used for the experiment. The rabbits were divided equally into 11 groups, and then bilateral abdomen area of each rabbit was shaved. Group 1 (n = 5) consisted of untreated controls (0.9% NaCl 2 mL p.o); groups 2, 3, and 4 (n =5, each group) were treated with amlodipine (1, 3, and 6 mg kg^{-1} p.o. using gavages). Groups 5, 6, and 7 (n =5, each group) were treated with lacidipine (1, 3, and 6 mg kg^{-1} p.o. using gavages). Groups 8, 9, and 10 (n =5, each group) were treated with nicardipine (1, 3, and 6 mg kg^{-1} p.o. using gavages). Group 11 was treated with indomethacin (6 mg kg^{-1} p.o. using gavages). All drugs were dissolved in normal saline (2 mL 0.9% NaCl). Indomethacin was used to compare the effects of CCBs. 128 IU of hyaluronidase was dissolved in 1 mL of isotonic NaCl solution; 0.8 mL of trypan blue (0.75%) was added to 0.5 ml of this solution. The resulting mixture (0.1 mL) was injected subcutaneous into the abdominal shaved region 1 h after each drug administration. The appearance of the blue region was measured at 1 and 5 minutes after injection as mm^2 on shaved rabbit's abdomen area. In addition, diameters of the blue regions were also measured in the 30^{th} minute. The size of the blue area showed the activity of the hyaluronidase enzyme, and capillary vascular permeability. Reduced size of the blue area indicated lower activity of the hyaluronidase enzyme, and capillary vascular permeability (Suleyman *et al*., 2007). The effect of drugs on capillary permeability was evaluated by comparing the results of the test groups with those of the control group.

Intravenous hyaluronidase test

In this series of experiments, high effective dose of amlodipine (6 mg kg^{-1}), lacidipine (6 mg kg^{-1}), nicardipine (6 mg kg^{-1}) and indomethacin (6 mg kg^{-1}) on hyaluronidase activity was investigated with the hyaluronidase-induced capillary permeability test; 30 albino rabbits were used for the experiment. All drugs were given with per-oral. The control group received an equal volume of 0.9% NaCl as a vehicle. 128 IU of hyaluronidase was dissolved in 1 mL of isotonic NaCl solution and 0.1 mL kg^{-1} hyaluronidase was injected intravenous into the rabbit's ear vein one hour after drug administration. 30 minutes after hyaluronidase administration, trypan blue (0.75%) (0.1 mL) was injected subcutaneous into the abdominal shaved region. After the administration of the trypan blue, the effects of drugs on capillary permeability were determined as described above (Suleyman *et al*., 2007).

Statistical analyses

All results were shown as means ± SD. One way analysis of variance with post-hoc LSD test was used to evaluate the results; $P<0.05$ was accepted as the level of statistical significance.

RESULTS

Carrageenan-induced paw oedema

As shown in Table I and Fig. 1, amlodipine inhibited carrageenan-induced paw oedema at doses of 1, 3, and 6 mg kg^{-1} by 27.3, 43.7, and 67.3% four hour after carrageenan administration. Lacidipine inhibited carrageenan-induced paw oedema at doses of 1, 3, and 6 mg kg^{-1} by 37.1, 55.6, and 76.4% four hour after carrageenan administration. Nicardipine inhibited carrageenan-induced paw oedema at doses of 1, 3, and 6 mg kg^{-1} by 11.2, 31.0, and 91% four hour after carrageenan administration. The

Table I. Effects of amlodipine, lacidipine, nicardipine, and indomethacin on carrageenan-induced paw oedema in rats. Three doses of amlodipine, lacidipine, nicardipine and single dose of indomethacin treated groups were compared with carrageenan group.

Treatment	Number of animals	Dose mg kg^{-1} body wt.	Paw volume before inflammation (mL)	Paw volume after 4 hour inflammation (mL)	Difference between paw volumes (mL) at 4 h	Antiinflammatory effect Inhibition %
Amlodipine	6	1	0.84	1.24	0.40±0.11	27.3
Amlodipine	6	3	0.87	1.18	0.31±0.08*	43.7
Amlodipine	6	6	0.78	0.96	0.18±0.06**	67.3
Lacidipine	6	1	0.89	1.23	0.34±0.11*	37.1
Lacidipine	6	3	0.75	0.99	0.24±0.07*	55.6
Lacidipine	6	6	0.79	0.92	0.13±0.09**	76.4
Nicardipine	6	1	0.91	1.39	0.48±0.22	11.2
Nicardipine	6	3	0.82	1.20	0.38±0.14*	31.0
Nicardipine	6	6	1.01	1.06	0.05±0.003**	91.0
Indomethacin	6	6	0.97	1.31	0.34±0.11*	38.2
Control	6	-	0.86	1.41	0.54±0.09	-

*Significant at $p<0.05$; **Significant at $p<0.01$.

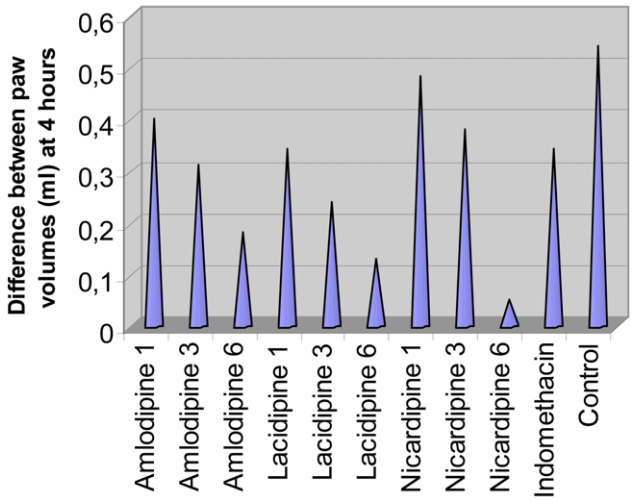

Fig. 1. Effects of amlodipine, lacidipine, nicardipine, and indomethacin on carrageenan-induced paw oedema in rats (Difference between paw volumes (mL) after 4 h carregeenan injection)

inhibition resulting from indomethacin was found to be 38.2%. Significant increases of inflammatory paw volumes resulted from amlodipine at doses of 3 and 6 mg kg^{-1} ($P<0.02$ and $P<0.001$, respectively), lacidipine at doses of 1, 3, and 6 mg kg^{-1} ($P<0.05$, $P<0.02$ and $P<0.001$ respectively), nicardipine at doses of 3 and 6 mg kg^{-1} ($P<0.05$ and $P<0.0001$, respectively) and indomethacin at a dose of 6 mg kg^{-1} ($P<0.05$), but not from amlodipine 1 mg kg^{-1} and nicardipine 1 mg kg^{-1} ($P>0.05$).

Hyaluronidase test

As seen in Table II and Fig. 2, the subcutaneous spreading area of trypan blue after 1 minute of subcutaneous injection of hyaluronidase was 153±14.5 mm^2 in the control group, while it was 157±7.2, 162±14.7, and 155±18.3 at the dose of amlodipin 1, 3, and 6 mg kg^{-1} respectively. The subcutaneous spreading area of trypan blue after 1 minute of subcutaneous injection of hyaluronidase was 139±11.7, 123±8.9, and 141±11.0 mm^2 at the dose of

Table II. Effects of amlodipine, nicardipine, lacidipine, and indomethacin to hyaluronidase-induced capillary vascular permeability. Three doses of amlodipine, lacidipine, nicardipine and single dose of indomethacin treated groups were compared with control group.

Drugs	Number of rabbits	Dose (mg kg^{-1})	Spreading area of hyaluronidase + trypan blue mixture (mm^2)		
			After 1 minute	After 5 minute	After 30 minute
Amlodipine	5	1	157 ± 7.2	244±37	411 ±83
Amlodipine	5	3	162 ±14.7	201±47.3*	333 ±56*
Amlodipine	5	6	155 ±18.3	177±29*	311 ±25.3*
Lacidipine	5	1	139 ±11.7	191±13.5*	357 ±55.6*
Lacidipine	5	3	123 ± 8.9	193±13.1*	336 ±19.2*
Lacidipine	5	6	141 ±11.0	164±14.3*	251 ±12.9*
Nicardipine	5	1	170 ±18.3	287±31	495 ±63
Nicardipine	5	3	164 ±11.7	219±27*	388 ±29*
Nicardipine	5	6	97.3± 4.7*	126± 7.3*	197 ±17.3**
Indomethacin	5	6	151 ± 3.7	223± 4.9*	394 ±22.9*
Control	5	-	153 ±14.5	313±39	507.8±87

*Significant at $p<0.05$; **Significant at $p<0.01$.

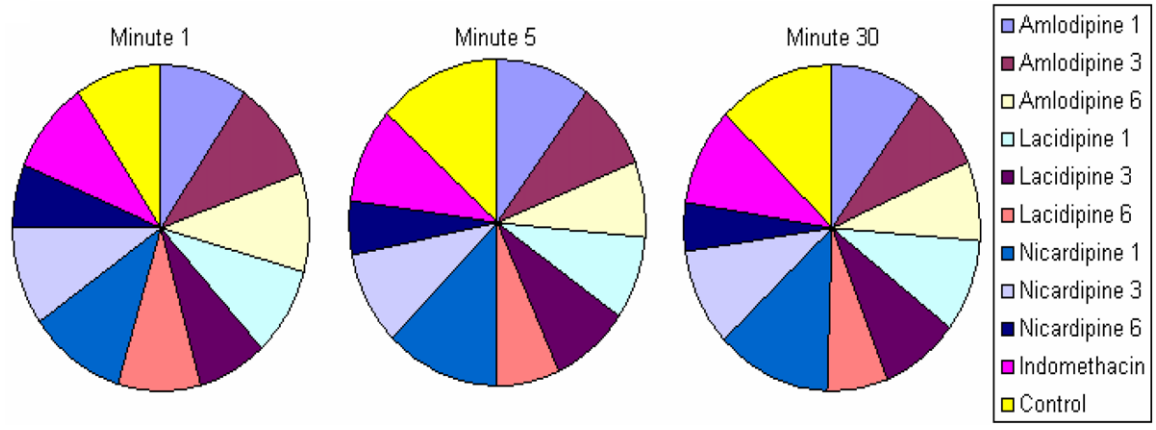

Fig. 2. Effects of amlodipine, nicardipine, lacidipine, and indomethacin to hyaluronidase-induced capillary vascular permeability (Spreading area of hyaluronidase + trypan blue mixture)

lacidipine 1, 3, and 6 mg kg^{-1} while 170±18.3, 164±11.7 and 97.3±4.7 mm^2 at the dose of nicardipine 1, 3, and 6 mg kg^{-1} respectively. The subcutaneous spreading area of trypan blue after 5 minute of subcutaneous injection of hyaluronidase was 313±39 mm^2 in the control group, while it was 244±37, 201±47.3, and 177±29 at the dose of amlodipin 1, 3, and 6 mg kg^{-1} respectively. The subcutaneous spreading area of trypan blue after 5 minute of subcutaneous injection of hyaluronidase was 191±13.5, 193±13.1, 164±14.3 mm^2 at the dose of lacidipine 1, 3 and 6 mg kg^{-1} while 287±31, 219±27 and 126±7.3 mm^2 at the dose of nicardipine 1, 3 and 6 mg kg^{-1} respectively. The subcutaneous spreading area of trypan blue after 30 minute of subcutaneous injection of hyaluronidase was 507.8±87 mm^2 in the control group, while it was 411±83, 333±56, and 311±25.3 at the dose of amlodipin 1, 3, and 6 mg kg^{-1} respectively. The subcutaneous spreading area of trypan blue after 30 minute of subcutaneous injection of hyaluronidase was 357±55.6, 336±19.2, and 251±12.9 mm^2 at the dose of lacidipine 1, 3, and 6 mg kg^{-1} while 495±63, 388±29, and 197±17.3 mm^2 at the dose of nicardipine 1, 3, and 6 mg kg^{-1} respectively. 30 minutes after enzyme administration, amlodipine at 3 and 6 mg kg^{-1} doses inhibited the spreading area significantly (P<0.05), but not at 1 mg kg^{-1} dose (P>0.05). In the lacidipine group, with doses of 1, 3, and 6 mg kg^{-1}, the diameter of the blue area was significantly smaller (P<0.05, P<0.05 and P<0.02 respectively) (Table II). Also, nicardipine at 3 and 6 mg kg^{-1}, inhibited the spreading area significantly (P<0.05 and P<0.01), but not at 1 mg kg^{-1} dose (P>0.05) (Table II). In the indomethacin group (6 mg kg^{-1}), the diameter of blue area was also significantly smaller both at 5^{th} and 30^{th} minutes (P<0.05) (Table II).

Intravenous hyaluronidase test

As seen in Table III and Fig. 3, subcutaneous speading area of trypan blue resulting from IV administration of hyaluronidase enzyme was 136.6±6.5, 210.3±25.8, and 335.6±57.2 mm^2 in the control group after 1, 5, and 30 minutes respectively. Subcutaneous speading area of trypan blue resulting from IV administration of hyaluronidase enzyme was 134.3±12.5, 161.8±18.6, and 188.8±32.1 mm^2 in the amlodipine group, with the dose of 6 mg kg^{-1} after 1, 5, and 30 minutes respectively. In the lacidipine group, with the dose of 6 mg kg^{-1}, the diameter of the blue area was 140.8±6.3, 165.1±12.4, and 198.6±22.41 respectively (Table III). In the nicardipine group, with the dose of 6 mg kg^{-1}, the diameter of the blue area was 139.8±7.8, 150±4.1, and 157.5±3.5 respectively (Table III). In the indomethacin group (6 mg kg^{-1}), the diameter of blue area was 138.5±9.6, 158.5±14.5, and 215.8±22.7 respectively (Table III).

Table III. Effects of amlodipine, nicardipine, lacidipine, and indomethacin to IV hyaluronidase-induced capillary vascular permeability. Single dose of amlodipine, lacidipine, nicardipine, and indomethacin treated groups were compared with control group.

Drugs	Number of rabbits	Dose (mg kg^{-1})	Spreading area of trypan blue (mm^2)		
			After 1 minute	After 5 minute	After 30 minute
Amlodipine	6	6	134.3±12.5	161.8±18.6*	188.8±32.1*
Lacidipine	6	6	140.8± 6.3	165.1±12.4*	198.6±22.41*
Nicardipine	6	6	139.8± 7.8	150 ± 4.1*	157.5± 3.5*
Indomethacin	6	6	138.5± 9.6	158.5±14.5*	215.8±22.7*
Control	6	-	136.6± 6.5	210.3±25.8	335.6±57.2

*Significant at p<0.05.

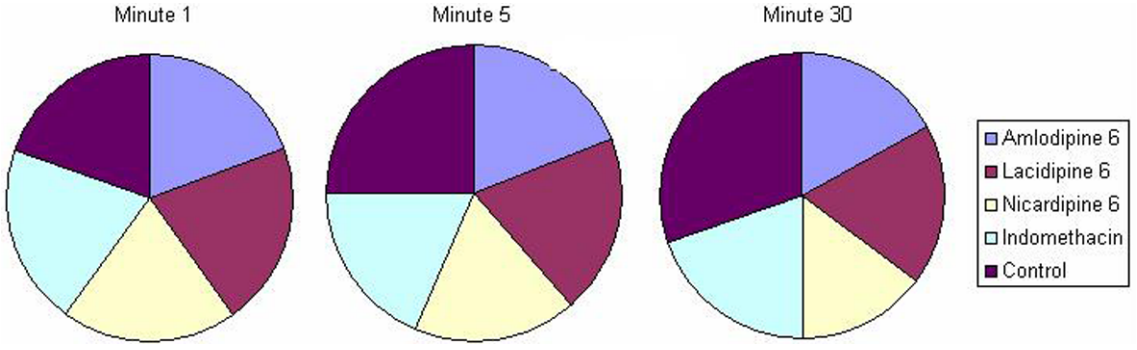

Fig. 3. Effects of amlodipine, nicardipine, lacidipine, and indomethacin to IV hyaluronidase-induced capillary vascular permeability (Spreading area of trypan blue)

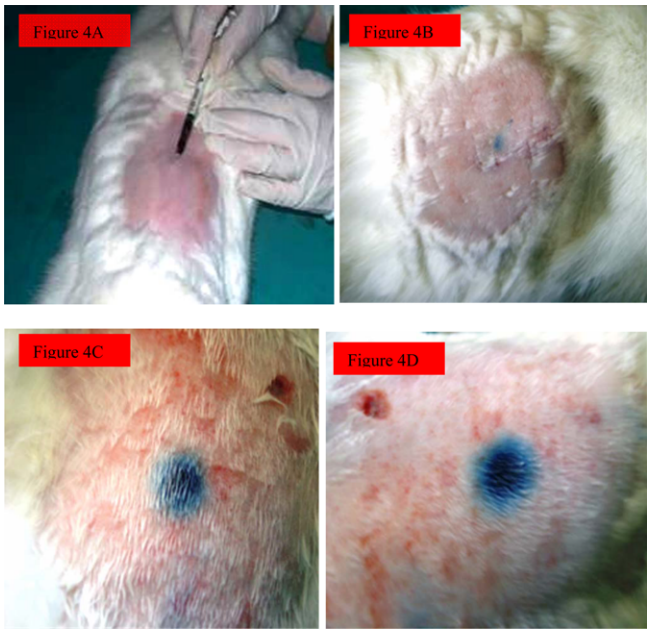

Fig. 4. Subcutaneous injection of trypan blue (4A). Spreading areas of trypan blue (4B, 4C and 4D)

DISCUSSION

The effects of amlodipine, lacidipine, and nicardipine were investigated in the acute phase of inflammation in the carrageenan inflammation model, and the effect of these drugs on hyaluronidase activity was investigated with the hyaluronidase-induced capillary permeability test. In one of our previous studies we showed that amlodipine and lacidipine clearly prevented carrageenan-induced inflammation at 5 and 10 mg kg^{-1} doses (Suleyman *et al*., 2006). The anti-inflammatory activities of amlodipine, lacidipine, nicardipine and indomethacin were investigated against experimental models of acute inflammation: paw oedema in rats, at doses of 1, 3, and 6 mg kg^{-1} in the first series of our experiment. We studied our drugs at 1, 3, and 6 mg kg^{-1} doses in order to determine whether the anti-inflammatory efficacy of CCBs is in line with decreased capillary permeability. The results of our experiments showed that anti-inflammatory effects of CCBs increase in a dose dependent manner, but the anti-inflammatory effects of amlodipine and nicardipine were not statistically significant at a dose of 1 mg kg^{-1}.

Our studies on rats showed that all CCBs which we tested, clearly prevented carrageenan-induced inflammation at doses of 3 and 6 mg kg^{-1}; 3 mg kg^{-1} doses of amlodipine and lacidipine have a more significant anti-inflammatory effect than indomethacin; 6 mg kg^{-1} doses of amlodipine, lacidipine and nicardipine were, respectively, 1.7, 2, and 2.3 times more effective compared with indomethacin. This dose of CCBs inhibited carrageenan inflammation significantly when compared not only with control, but also with indomethacin groups. Indomethacin was shown to have higher effects at 20-25 mg kg^{-1} doses, but lower effects at 10 mg kg^{-1} dose (Suleyman *et al*., 1998; 1999; 2003). The excessive production of hydrogen peroxide (H_2O_2), O_2^-, and hydroxyl (OH^-) radicals, the activation of polymorph nuclear leucocytes (PNL) and metabolites of arachidonic acid, and the increase in the synthesis of inflammation mediators such as histamine, serotonin, bradykinin, and NO are known to have an important role in the pathogenesis of carrageenan induced inflammation (Salomone *et al*., 1996; Marzocco *et al*., 2004; Ramprasath *et al*., 2004; Neto *et al*., 2005). On the other hand, these inflammatory processes are mediated by impairment in calcium homeostasis; there has been interest in the potential role of CCBs as anti-inflammatory agents (Henry, 1985; Henry and Bentley, 1989). Many studies show the important role of calcium ions on the synthesis and release of these inflammation mediators, which are present in the pathogenesis of carrageenan induced inflammation (Elferink *et al*., 1992; Leslie, 1997; Carnevale and Cathcart, 2001; El-Bizri *et al*., 2003). The anti-inflammatory mechanisms of CCB can be summarized as inhibition of the synthesis of the products of cyclooxygenase and lipoxygenase as non-steroidal anti-inflammatory drugs (NSAIDs), prevention of aggregation, adhesion and chemotaxis of neutrophils, blockage of the release of lysosomal enzymes and toxic oxygen radicals, and uncoupling of oxidative phosphorylation (Martinez *et al*., 1999; Sirmagul *et al*., 2004; Kouoh *et al*., 2006). A review of the scientific literature provides support for several anti-atherosclerotic mechanisms that may be involved in the benefits of third-generation CCBs in cardiovascular disease; these explained the anti-inflammatory mechanism (Tulenko *et al*., 1995; Kramsch, 1997; Tulenko *et al*., 1997; Mason, 1998; Mason *et al*., 1999).

In the second series of experiments, the effects of amlodipine, lacidipine, nicardipine, and indomethacin on hyaluronidase activity was investigated with the hyaluronidase-induced capillary permeability test. Results obtained showed a relationship between anti-inflammatory action and hyaluronidase enzyme inhibition. The most effective drug in carrageenan inflammation, nicardipine (6 mg kg^{-1}), also had the most significant effect according to the hyaluronidase test. Amlodipine, lacidipine, and indomethacin were unable to decrease the spreading area of hyaluronidase significantly at the 1st minute. All doses of nicardipine used decreased the spreading area of hyaluronidase significantly at 5 and 30 minutes. Doses of 10 and 25 mg kg^{-1} of indomethacin which inhibited carrageenan inflammation significantly were also able to reduce the hyaluronidase induced increase in capillary permeability significantly, in comparison to control. Ineffective doses of amlodipine (1

mg kg^{-1}) and nicardipine (1 mg kg^{-1}) on inflammation also failed to decrease the spreading area of hyaluronidase at all minutes measured. The most effective dose of amlodipine (6 mg kg^{-1}), lacidipine (6 mg kg^{-1}), nicardipine (6 mg kg^{-1}), and indomethacine were decreased the spreading area of hyaluronidase significantly at 5 and 30 minutes when hyaluronidase injected intravenously.

As we explained in the material and method section, the size of blue area shows activity of hyaluronidase enzyme and capillary vascular permeability. A smaller blue area shows that the activity of hyaluronidase enzyme and capillary vascular permeability are decreased (Suleyman *et al*., 2003). Capillary permeability increases with the effect of inflammation, for many reasons. Among these, hydrogens peroxide (H_2O_2) can be considered an initial cause. H_2O_2 is an oxidant released by PNL activation and plays an important role in PNL-induced increases in endothelial permeability (Siflinger-Birnboim *et al*., 1996). However excessive release of this compound causes many different types of pathologic damage. Endothelial permeability is among these, and is the one in which we are particularly interested. In a study performed by Yamada *et al*., H_2O_2 was shown to increase endothelial permeability; L type CCBs were shown to antagonise this effect of H_2O_2 (Yamada *et al*., 1990). The antioxidant effects of L type CCBs are well known (Chen *et al*., 1997). In many studies they have been shown to reduce reactive oxygen species (ROS) in inflammatory tissues. In atherosclerotic lesions, an increase in the production of reactive oxygen species together with inflammatory factors such as chemokines, cytokines, and adhesion molecules has been shown (Ross, 1999; Liao *et al*., 1994). Reduction of increased vascular permeability in either atherogenesis or inflammation by L type CCBs may be related to their antioxidative effects, resulting in decreased levels of many oxygen radicals, especially H_2O_2 (Yamada *et al*., 1990). Intracellular interactions, which regulate the endothelial cell-related vascular permeability, can be considered as another potential mechanism. Which is, vascular permeability increases in line with the increased intracellular calcium concentration. Increased calcium concentration activates protein kinaz G (Huang *et al*., 1997). There are several mechanisms related to increase calcium concentration. Among these histamines and bradikinine, which are the mediators which occur in acute inflammation; NO and ROS such as H_2O_2 can be considered as the first (Al-Naemi *et al*., 2000). Excess H_2O_2 increases extracellular calcium, which then enters endothelial cells, resulting in increased permeability (Siflinger-Birnboim *et al*., 1996). CCBs may prevent the increase in endothelial permeability by inhibiting calcium's entrance to cells. CCBs have also been shown to inhibit histamin-induced inflammation (Bilici *et al*., 2001). Another mechanism for these effects of CCBs may be their direct preventive effect on the entrance of extracellular calcium to cells.

CONCLUSION

Our results showed that the hyaluronidase enzyme plays a role in the increased capillary permeability which occurs in the inflammatory response, as well as other inflammatory mediators. As mentioned above, significant inhibition of hyaluronidase activity by CCBs at the doses we studied, with significant anti-inflammatory effects, shows the role of the hyaluronidase enzyme in the anti-inflammatory action mechanism of these drugs.

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