The Role of Cyclooxygenase Enzymes in the Effects of Losartan and Lisinopril on the Contractions of Rat Thoracic Aorta

Losartan ve Lizinoprilin Sıçan Torasik Aort Kasılmaları Üzerindeki Etkilerinde Siklooksijenaz Enzimlerinin Rolü

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ABSTRACT

Objective: It was suggested that prostaglandins which are synthesized by cyclooxygenase (COX) enzymes contribute to the actions of angiotensin-converting enzyme (ACE) inhibition and angiotensin AT1 receptor antagonism and there is an interaction between ACE signaling pathway and COX enzymes. We aim to investigate the role of COX enzymes in the effects of losartan, an angiotensin II (Ang II) receptor antagonist or lisinopril, an ACE inhibitor, on the contractions of rat thoracic aorta in isolated tissue bath.

Materials and Methods: Responses of losartan (10^{-6}, 10^{-5}, 10^{-4} M), lisinopril (10^{-6}, 10^{-5}, 10^{-4} M), and non-selective COX inhibitor dipyrone (10^{-4}, 7×10^{-4}, 2×10^{-3} M) alone to the contractions induced by phenylephrine (Phe) (10^{-7} M), potassium chloride (KCl) (6×10^{-2} M), Ang II (10^{-8} M) and responses of losartan or lisinopril in combination with dipyrone to the contractions induced by Phe or KCl were recorded.

Results: When used alone, dipyrone and losartan inhibited Phe, KCl, and Ang II-induced contractions, whereas lisinopril inhibited only Phe and Ang II-induced contractions. Inhibition of COX enzymes (COX-3, COX-3 + COX-1, COX-1+ COX-2 + COX-3 by dipyrone 10^{-4}, 7×10^{-4}, 2×10^{-3} M, respectively) augmented the relaxant effects of losartan or lisinopril. Also, dipyrone potentiated the effect of lisinopril on KCl-induced contractions.

Conclusion: We suggest that dipyrone increases the smooth-muscle relaxing effects of losartan or lisinopril and that COX enzyme inhibition may have a role in the enhancement of this relaxation.

Keywords: Cyclooxygenase, dipyrone, lisinopril, losartan, thoracic aorta

ÖZ

Amaç: Siklooksijenaz (COX) enzimleri tarafından sentezlenen prostaglandinlerin anjiotensin dönüştürücü enzim (ADE) inhibisyonu ve anjiotensin AT1 reseptör antagonizmasının etkilerine katkıda bulunduğu ve ADE sinyal yolakları ile COX enzimleri arasında etkileşme olduğu ileri sürülmüştür. Bu çalışmada anjiotensin II (Ang II) reseptör antagonisti bir ilaç olan losartan veya ADE inhibitörü bir ilaç olan lizinoprilin izole organ banyosunda sıçan torasik aorta kasılmaları üzerindeki etkilerinde COX enzimlerinin rolünün araştırılmasını amaçladık.

Gereç ve Yöntem: Losartan (10^{-6},10^{-5},10^{-4}M), lizinopril (10^{-6},10^{-5},10^{-4}M) ve selektif olmayan bir COX inhibitörü olan dipironun (10^{-4},$7x10^{-4}$,$2x10^{-3}$M) tek başına fenilefrin (Phe) (10^{-7}M), potasyum klorür (KCl) ($6x10^{-2}$M) ve Ang II (10^{-8}M) ile indüklenen kasılmalar üzerindeki ve ayrıca losartan veya lizinoprilin dipironla kombinasyonlarının Phe veya KCl ile indüklenen kasılmalar üzerindeki yanıtları kaydedildi.

Bulgular: Tek başlarına verildiklerinde dipiron ve losartan Phe, KCl ve Ang II ile indüklenen kasılmaları baskılarken, lizinopril sadece Phe ve Ang II ile indüklenen kasılmaları baskıladı. COX enzimlerinin inhibisyonu (dipiron 10^{-4},$7x10^{-4}$,$2x10^{-3}$M tarafından sırasıyla COX-3, COX-3+-1, COX1+-2+3), losartan veya lizinoprilin gevşetici etkilerini artırdı. Ayrıca dipiron, lizinoprilin KCl ile indüklenen kasılmalar üzerindeki etkisini potansiyalize etti.

Sonuç: Dipironun losartan veya lizinoprilin düz kas gevşetici etkilerini artırdığını ve COX enzim inhibisyonunun bu gevşemede rolü olabileceğini ileri sürüyoruz.

Anahtar Kelimeler: Siklooksijenaz, dipiron, lisinopril, losartan, torasik aorta

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Introduction

Angiotensin II (Ang II) regulates and maintains physiological vascular tone and function. However, increase of Ang II is involved in pathological processes such as hypertension [1]. Most of the effects of Ang II are associated with the AT_1 receptors, and Ang II generates contraction by activating AT_1 receptors which are selectively blocked by AT_1 receptor antagonists [2].

Targeting the renin-angiotensin-aldosterone axis is focused to cure hypertension including renin inhibitors, angiotensin-converting enzyme (ACE) inhibitors, and angiotensin II receptor blockers

[3]. The antihypertensive effect elicited by ACE inhibition is reported to involve a reduction in the levels of Ang II, an increase in tissue kinin concentrations, as well as the release of nitric oxide and prostanoids [4].

Prostanoids are considered as important cardiovascular regulatory mediators. They play significant actions for controlling physiological vascular tonus, renin release, and blood pressure [5]. Cyclooxygenase (COX) enzymes which are inhibited by non-steroidal anti-inflammatory drugs (NSAIDs) catalyze the rate-limiting step of prostanoid synthesis [6]. Up to date, three COX enzymes have been defined: COX-1, -2, and -3 [7]. It was reported that COX-1 is constitutive and responsible for the generation of prostaglandins which mediate a number of physiological effects, whereas COX-2 is induced in multiple tissues in conditions such as inflammation [8]. Recently, COX-3 has been defined in canine, rodents, and humans [7, 9]. Although the expression of COX-3 was strongest in heart, kidney, and neuronal tissues, different rat tissues including aorta showed COX-3 expression [10]. Most of the prostanoids are formed by COX-1 and are involved in physiologic responses [8]. COX-2-mediated prostanoid production is also responsible for the regulation of vascular functions and physiologic and pathophysiologic processes, such as renal hemodynamics, control of blood pressure, and endothelial thromboresistance [11]. On the other hand, there is not enough data on the clinical relevance of COX-3 in humans [12].

The evidence shows that there is an association between Ang II and COX expression. Ang II induces COX-2 in vascular and non-vascular tissues [1]. It was reported in smooth-muscle cells that the production of COX-2-mediated prostanoids contributes to the short-lasting actions of Ang II and that the participation of COX-2-derivated prostanoids did not differ between abdominal and thoracic aorta [13]. However, endothelium inhibited COX-2 mRNA expression in the thoracal but not in the abdominal aorta, whereas the involvement of the endothelium-independent COX-1 in the contractive effect of Ang II occurred in the abdominal but not in the thoracal aorta [13]. Ang II was proposed to regulate COX-2 expression and prostanoid formation in vascular smooth muscle via AT_1 receptors [14]. In addition, an AT_1 receptor antagonist losartan decreased COX-2 expression in renal tissue [15].

An interaction between angiotensin-converting enzyme (ACE) signaling pathway and COX enzymes has also been suggested [16]. Although the effects of ACE inhibitors (ACE-I) are generally referred to the reduction of Ang II and the increase of bradykinin, some effects of ACE-Is are thought to be unallied with ACE inhibition. It was reported that ACE-Is increased COX-2 expression as well as the production of the vasodilator prostacyclin via ACE signaling pathway [16]. However, it was found that enalapril, an ACE inhibitor, did not increase COX-2 expression [17]. Non-selective inhibition of COX or selective inhibition of COX-2 diminished the antihypertensive effect of ACE-Is [17]. Furthermore, COX-2 inhibitors such as rofecoxib, celecoxib, and nimesulide reversed the thrombolytic effects induced by ACE-Is [18].

Cyclooxygenase enzymes are inhibited by some of the NSAIDs selectively in a concentration dependent manner [7]. Dipyrone is a member of NSAIDs with analgesic and antipyretic features. It has spasmolytic and vascular smooth-muscle relaxing effects which differentiate it from the other NSAIDs [19]. It inhibits COX-3 more potently at low concentrations (IC_{50}: 52 μM), whereas it inhibits COX-3 + COX-1 at moderate (IC_{50}: 350 μM) and COX-3 + COX-1 + COX-2 at high (IC_{50} > 1000 μM) concentrations, respectively [7].

We aimed to investigate the role of COX enzymes by using the non-selective COX inhibitor dipyrone, in the effects of an AT_1 receptor blocker, losartan and, an ACE-I, lisinopril on the contractile activity on isolated rat thoracic aorta.

Materials and Methods

Animals

Male Spraque-Dawley rats (200-250 g) were housed in standard laboratory conditions. Food and water was supplied ad libitum. The experiments were executed with the approval of the Local Ethics Committee for the Care and Use of Experimental Animals of Eskisehir Osmangazi University in accordance with the Guide for the Care and Use of Laboratory Animals.

Chemicals and drugs

In this study, dipyrone (Sigma-Aldrich Inc., Germany), phenylephrine hydrochloride (Phe) (Sigma-Aldrich Inc., Germany), acetylcholine chloride (ACh) (Sigma-Aldrich Inc., Germany), potassium chloride (KCl) (Sigma-Aldrich Inc., Germany), angiotensin II (Ang II) human (Sigma-Aldrich Inc., Germany), losartan potassium (Sanovel Pharmaceuticals, Turkey), and lisinopril dihydrate (Sanovel Pharmaceuticals, Turkey) were used. We preferred to use lisinopril as an ACE-I because of its feature being not a pro-drug that is suitable to use for an in vitro experiment.

Preparation of rat aortas

Rats were sacrificed by cervical dislocation under light ether anesthesia. The descending thoracic aorta was rapidly dissected out and immersed in Krebs solution (in millimoles per liter) composed of NaCl: 118; KCl: 4.7; $MgSO_4$: 1.2; KH_2PO_4: 1.2; glucose: 11.1; $NaHCO_3$: 24.9 and $CaCl_2$: 2.5. After removing the perivascular tissue, aortic rings in approximately 4-mm length were cut. Aortic rings were suspended in organ baths containing 10-mL Krebs solution at 37°C and aerated with 95% O_2 and 5% CO_2. The rings were maintained to equilibrate for 1 hour at a resting tension of 1 g which was determined by length-tension relationship experiments. During the equilibration period, Krebs solution in the baths was changed every 15 minutes. The tension of the rings was recorded by isometric transducers connected to a data acquisition system and amplifier (MAY TDA 96 Transducer data acquisition system; MAY 96-BA Bridge amplifier, Commat Pharmacology and Physiology Instruments, Ankara/Turkey). Functional integrity of the endothelium was verified qualitatively by the degree of relaxation caused by ACh (10^{-6} M) in the presence of contractile tone induced by Phe (10^{-7} M). The rings were discarded if relaxation with ACh was not 80% or greater. In the experimental protocol, the relaxation percentage was evaluated as the contraction after the administration of dipyrone, losartan, and lisinopril in the presence of the contracting agents Phe, KCl, and Ang II.

Experimental protocol

We performed three series of experiments. First, we investigated the concentration-dependent effects of dipyrone on Phe, KCl, and Ang II-induced contractions. Accordingly, aortic rings were precontracted with Phe (10^{-7} M), KCl (6×10^{-2} M), and Ang II (10^{-8} M). Then, aortic rings were incubated with dipyrone (10^{-4}, 7×10^{-4}, and 2×10^{-3} M) for 15 minutes, and the percentages of the contractions were calculated. The concentrations of dipyrone were chosen twice-fold of IC_{50} values which are expected to inhibit COX-3, COX-3 + COX-1, and COX-3 + COX-1 + COX-2, respectively [7, 20].

Second, the concentration-dependent effects of losartan and lisinopril on Phe, KCl, and Ang II-induced contractions were determined. Losartan (10^{-6}, 10^{-5}, 10^{-4} M) or lisinopril (10^{-6}, 10^{-5}, 10^{-4} M) were added after contractions induced by Phe (10^{-7} M), KCl (6×10^{-2} M), or Ang II (10^{-8} M).

Finally, we studied the concentration-dependent effects of dipyrone in the effects of losartan and lisinopril in response to Phe and KCl-induced

contractions. At first, the aortic preparations were precontracted with Phe (10^{-7} M) or KCl (6×10^{-2} M). Then, losartan (10^{-6}, 10^{-5}, 10^{-4} M) or lisinopril (10^{-6}, 10^{-5}, 10^{-4} M) were added to the rings in baths before the administrations of dipyrone at different concentrations (10^{-4}, 7×10^{-4}, and 2×10^{-3} M).

Statistical analysis

A paired or unpaired Student's t test or analysis of variance was used to analyze the data when applicable. The results are given as mean ± SEM. p values of less than 0.05 were accepted as statistically significant.

Results

Effects of dipyrone on Phe, KCl, or Ang II-induced contractions

The effects of three concentrations of dipyrone (10^{-4}, 7×10^{-4}, and 2×10^{-3} M) on Phe (10^{-7} M), KCl (6×10^{-2} M), or Ang II (10^{-8} M)-induced contractions were studied. We observed that dipyrone showed a relaxation on the aortic rings precontracted with Phe, KCl, or Ang II (Figure 1). The relaxant effects of dipyrone on KCl and Ang II-induced contractions were concentration-dependent, whereas the inhibition on Phe-induced contractions was more at low (10^{-4} M) and high (2×10^{-3} M) concentrations than the moderate (7×10^{-4} M) concentration of dipyrone (Figure 1). Dipyrone, at concentrations of 10^{-4} and 2×10^{-3} M, relaxed Phe-induced contractions more than on KCl-induced contractions (Figure 1). Dipyrone 2×10^{-3} M also showed a higher inhibition on Ang II-induced contractions than KCl-induced contractions (Figure 1).

Effects of losartan or lisinopril on Phe, KCl, or Ang II-induced contractions

The effects of three different concentrations of losartan (10^{-6}, 10^{-5}, 10^{-4} M) and lisinopril (10^{-6}, 10^{-5}, 10^{-4} M) were investigated on Phe (10^{-7} M), KCl (6×10^{-2} M), and Ang II (10^{-8} M)-induced contractions. Losartan inhibited Phe, KCl, and Ang II-induced contractions in a concentration-dependent manner (Figure 2). Lisinopril also inhibited Phe and Ang II-induced contractions but did not inhibit the contractions induced by KCl (Figure 3). The relaxant effect of lisinopril on Ang II-induced contractions was concentration-dependent manner whereas its inhibitions of Phe-induced contractions were more at low (10^{-6} M) and high (10^{-4} M) concentrations than the moderate (10^{-5} M) concentration (Figure 3).

Effects of dipyrone in combination with losartan or lisinopril on Phe or KCl-induced contractions

Separate experiments were performed to evaluate the effects of three concentrations

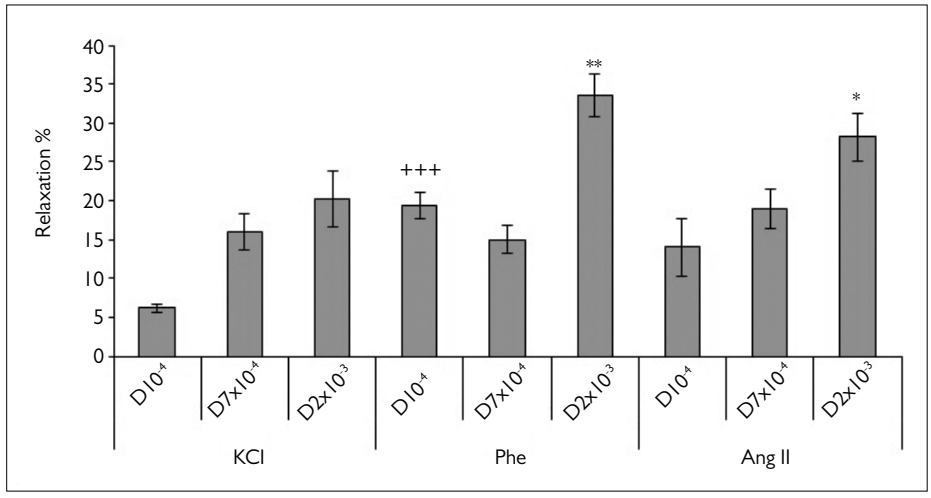

Figure 1. Effects of dipyrone on KCl, Phe, and Ang II-induced contractions. The data are expressed as mean ± SEM. *: p<0.05, compared with KCl + D 2×10^{-3}; **: p<0.01, compared with KCl + D 2×10^{-3}; +++: p<0.001, compared with KCl + D 10^{-4}. Phe: 10^{-7} M, KCl: 6×10^{-2} M, Ang II: 10^{-8} M, D 10^{-4}: 10^{-4} M, D 7×10^{-4}: 7×10^{-4} M, D 2×10^{-3}: 2×10^{-3} M.

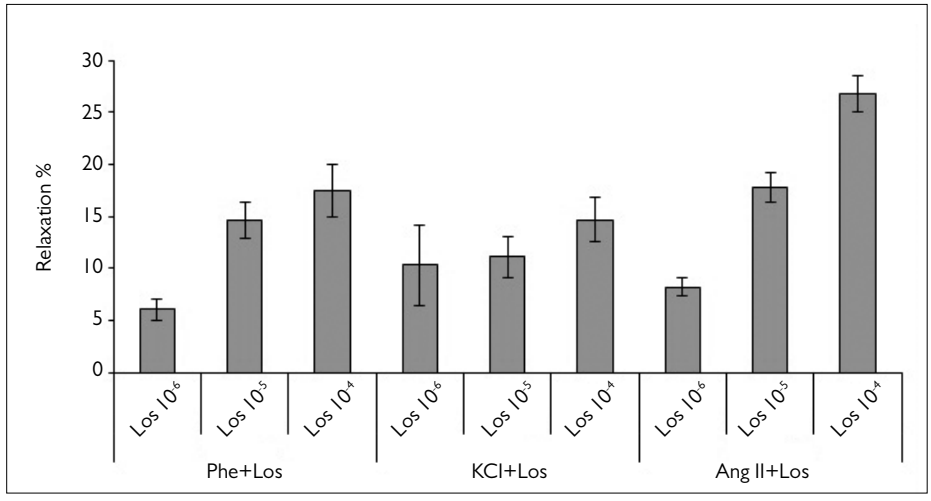

Figure 2. Effects of losartan on Phe, KCl, and Ang II-induced contractions. The data are expressed as mean ± SEM. Phe: 10^{-7} M, KCl: 6×10^{-2} M, Ang II: 10^{-8} M, Los 10^{-6}: 10^{-6} M, Los 10^{-5}: 10^{-5} M, Los 10^{-4}: losartan 10^{-4} M.

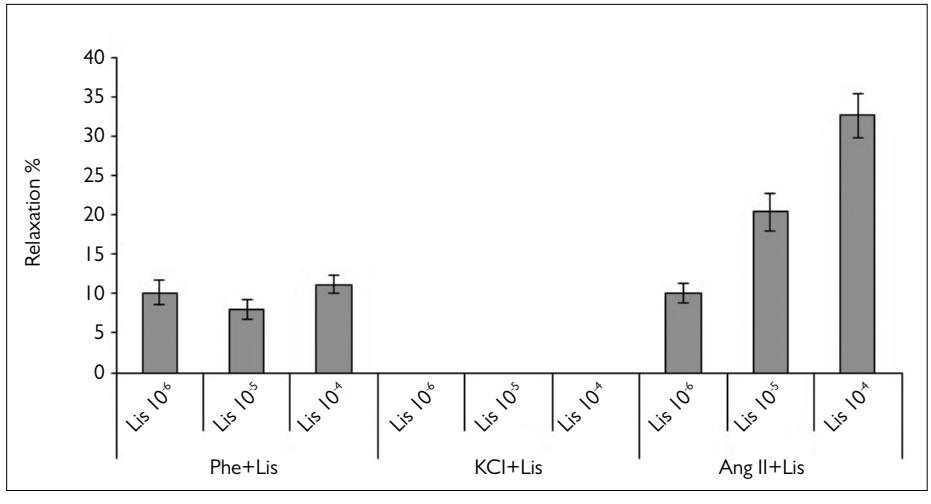

Figure 3. Effects of lisinopril on Phe, KCl, and Ang II-induced contractions. The data are expressed as mean ± SEM. Phe: 10^{-7} M, KCl: 6×10^{-2} M, Ang II: 10^{-8} M, Lis 10^{-6}: lisinopril 10^{-6} M, Lis 10^{-5}: lisinopril 10^{-5} M, Lis10^{-4}: lisinopril 10^{-4} M.

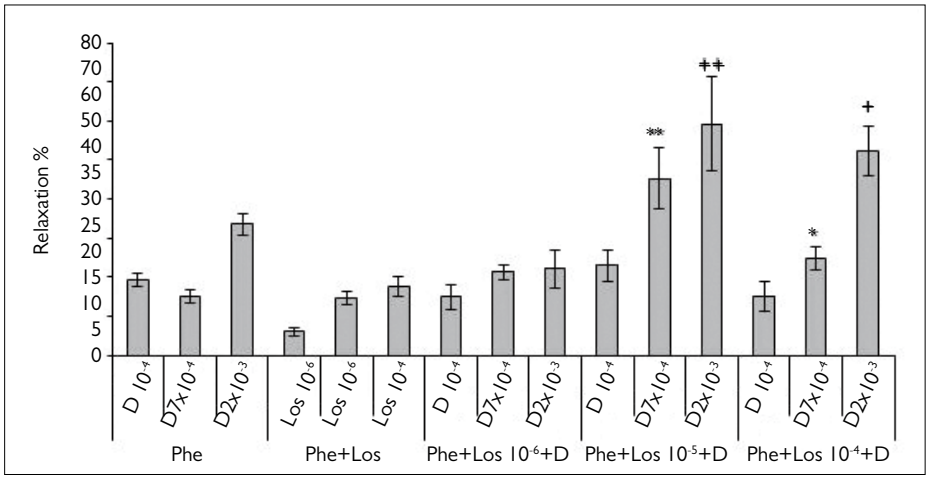

Figure 4. Effects of dipyrone and losartan combination on Phe-induced contractions. The data are expressed as mean ± SEM. *: p<0.05; **: p<0.01, compared with PE + D 7 × 10^{-4}; +: p<0.05; ++: p< 0.01 compared with PE + D 2 × 10^{-3}, Phe: 10^{-7} M, D 10^{-4}: 10^{-4} M, D 7 × 10^{-4}: 7 × 10^{-4} M, D 2 × 10^{-3}: 2 × 10^{-3} M, Los 10^{-6}: 10^{-6} M, Los 10^{-5}: 10^{-5} M, Los 10^{-4}: losartan 10^{-4} M.

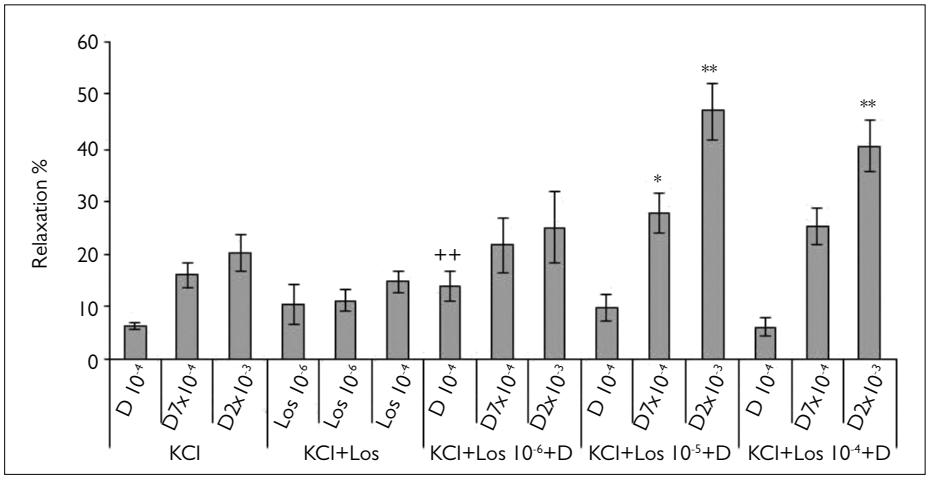

Figure 5. Effects of dipyrone and losartan combination on KCl-induced contractions. The data are expressed as mean ± SEM. *: p< 0.05, compared with KCl + D 7 × 10^{-4}; **: p<0.01, compared with KCl + D 2 × 10^{-3}; ++: p<0.01, compared with KCl + D 10^{-4}. KCl: 6 × 10^{-2} M, D 10^{-4}: dipyrone 10^{-4} M, D 7 × 10^{-4}: 7 × 10^{-4} M, D 2 x 10^{-3}: 2 x 10^{-3} M, Los 10^{-6}: 10^{-6} M, Los 10^{-5}: 10^{-5} M, Los 10^{-4}: 10^{-4} M.

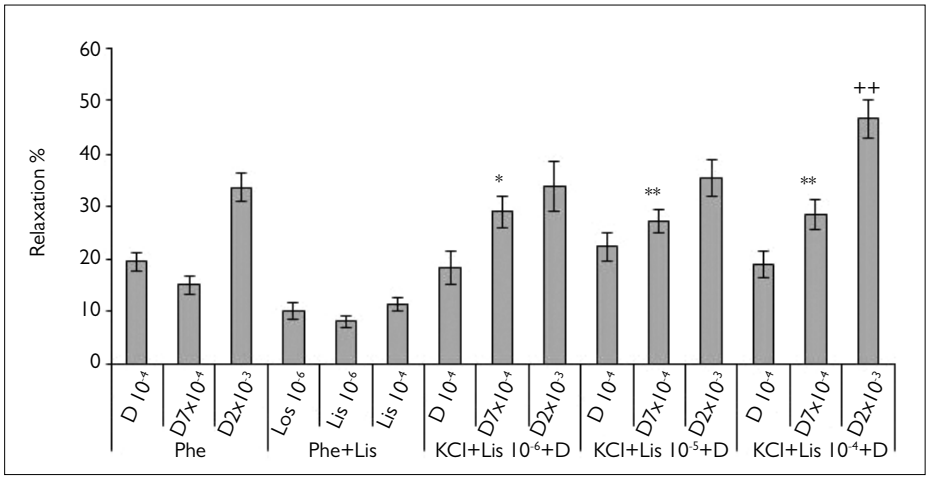

Figure 6. Effects of dipyrone and lisinopril combination on Phe-induced contractions. The data are expressed as mean ± SEM. *: p<0.05, compared with Phe + D 7 × 10^{-4}; **: p<0.01, compared with Phe + D 7 × 10^{-4}; ++: p<0.01, compared with PE + D 2 × 10^{-3}. Phe: 10^{-7} M, D 10^{-4}: 10^{-4} M, D 7 × 10^{-4}: 7 × 10^{-4} M, D 2 × 10^{-3}: D 2 × 10^{-3}M, Lis 10^{-6}: 10^{-6} M, Lis 10^{-5}: 10^{-5} M, Lis 10^{-4}: 10^{-4} M.

of losartan (10^{-6}, 10^{-5}, 10^{-4} M) and lisinopril (10^{-6}, 10^{-5}, 10^{-4} M) followed by three concentrations (10^{-4}, 7 × 10^{-4}, and 2 × 10^{-3} M) of dipyrone on Phe and KCl-induced contractions. Significant increases in relaxation percentage were observed with dipyrone 7 × 10^{-4} and 2 × 10^{-3} M and losartan 10^{-5} ($p<0.01$) and 10^{-4} M ($p<0.05$) combinations in response to Phe-induced contractions and also with dipyrone 10^{-4} M and losartan 10^{-6} M ($p<0.01$); dipyrone 7 × 10^{-4}, 2 × 10^{-3} M and losartan 10^{-5} M; dipyrone 2 × 10^{-3} M and losartan 10^{-4} M combinations on KCl-induced precontractions (Figure 4, 5). Similarly, significant enhancements in relaxation percentage were achieved with dipyrone 7 × 10^{-4} M ($p<0.05$) and lisinopril 10^{-6} M ($p<0.01$); dipyrone 7 × 10^{-4} M and lisinopril 10^{-5} M ($p<0.01$); dipyrone 7 × 10^{-4} and 2 × 10^{-3} M and lisinopril 10^{-4} M combinations in response to Phe-induced contractions ($p<0.01$) (Figure 6) and with dipyrone 10^{-4} M and lisinopril 10^{-6} M combinations on the contractions induced by KCl ($p<0.05$) (Figure 7).

Discussion

This research investigated whether the COX enzymes are involved in the effects of losartan or lisinopril on precontracted rat thoracic aorta in isolated tissue bath. In this regard, a COX inhibitor dipyrone, which is suggested to inhibit COX-3, COX-3 + COX-1, and COX-3 + COX-1 + COX-2 at low, moderate, and high concentrations, respectively, was used in combinations with losartan or lisinopril [7, 20].

We observed that dipyrone and losartan when used alone inhibited Phe, KCl, and Ang II-induced contractions, whereas lisinopril alone showed an inhibition only on Phe and Ang II-induced contractions. In addition, dipyrone augmented the relaxant effects of losartan on Phe- and KCl-induced contractions, augmented the relaxant effects of lisinopril on Phe-induced contractions, and potentiated the effect of lisinopril on KCl-induced contractions.

NSAIDs prevent prostaglandin synthesis by inhibiting COX enzymes [21]. Three isoforms of COX enzymes have been described [22]. The selectivity and potency of inhibiting COX isoforms differs among the NSAIDs. As an example, acetaminophen inhibits COX-3 more potently than COX-1 and COX-2 [9]. This mechanism of action can be extended to dipyrone, a pyrazolone derivate member of NSAIDs. Dipyrone inhibits COX-3 more potently at low concentrations (IC_{50}: 52 µM) whereas it inhibits COX-1 at moderate (IC_{50}: 350 µM) and COX-2 at high (IC_{50} > 1000 µM) concentrations, respectively, in cultured cells [7].

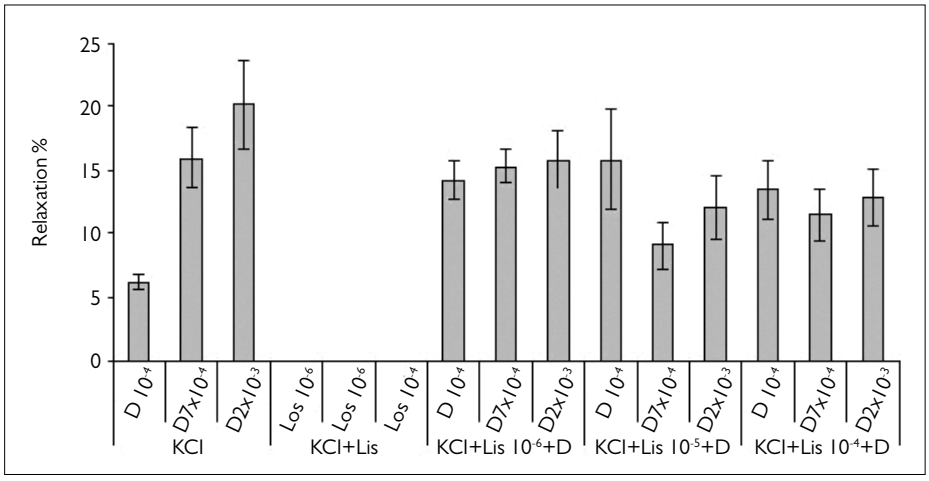

Figure 7. Effects of dipyrone and lisinopril combination on KCl-induced contractions. The data are expressed as mean ± SEM. KCl: 6×10^{-2} M, D 10^{-4}: 10^{-4} M, D 7×10^{-4}: 7×10^{-4} M, D 2×10^{-3}: 2×10^{-3} M, Lis 10^{-6}: 10^{-6} M, Lis 10^{-5}: 10^{-5} M, Lis 10^{-4}: 10^{-4} M.

Dipyrone is able to show relaxing effect on several tissues and also induces hypotension in humans at therapeutic doses [23]. The smooth-muscle relaxing and hypotensive effects of dipyrone impelled researchers to study its effects on vascular smooth muscle. Valenzuela et al. [24] stated that metamizol (dipyrone) inhibited Ang II-induced contractions on rat thoracal aorta concentration dependently.

This finding is completely well-matched with the results of our study in which dipyrone concentration dependently inhibited Ang II-induced contractions on rat thoracic aorta. Ergün et al. [25] observed that dipyrone concentration dependently inhibited Phe-induced contractions on rabbit thoracic aorta. This result is in agreement with the results of our study. Our results also demonstrated that dipyrone inhibited Phe-induced contractions. However, this effect was not in a concentration-dependent manner. Dipyrone showed more inhibition on Phe-induced contractions at low (10^{-4} M) and high (2×10^{-3} M) concentrations than the moderate (7×10^{-4} M) concentration which suggests a biphasic effect.

Loh et al. [26] studied the effects of losartan on Ang II-induced contractions in the aorta of the spontaneously hypertensive rats and observed that losartan abolished Ang II-induced contractions. In our study, we also observed that losartan inhibited Ang II-induced contractions. Santuzzi et al. [27] observed that losartan reduced Phe-induced contractions, similar to the results of our study. Accorsi-Mendonça et al. [28] observed that the ACE inhibitor trandolapril treatment decreased the Ang II- and Phe-induced but not KCl-induced contractions on isolated carotid arteries in rats. These results are partly in accordance with the results of our study. Although we performed our experiments on isolated rat thoracic aorta, we also observed that the ACE inhibitor lisinopril decreased the contractions induced by Phe or Ang II but not KCl-induced contractions. However, they performed the KCl experiments on the vascular tissue without endothelium while we used endothelium-intact aortic tissues.

It was reported that excessive contractile prostanoid formation by COX-2 was correlated with enhanced vascular contractility [29]. Alvarez et al. [30] investigated the effects of Ang II through AT_1 receptors and the contribution of prostanoids formed by COX-2 in the actions of Phe in rats. They concluded that Ang II increased COX-2-derived contractile prostanoids by AT_1 receptor activation in Phe-induced vasoconstriction, probably by the modulation of COX-2 expression. They also observed that losartan reduced COX-2 expression, and the selective COX-2 inhibitor NS-398 inhibited Phe-induced responses. In some aspects, these results support the results of our study. We used the AT_1 receptor antagonist losartan or the ACE inhibitor lisinopril combined with the COX inhibitor dipyrone. İnhibitory effects were enhanced with the concomitant use of dipyrone and losartan or lisinopril on Phe- and KCl-induced contractions. This may be a consequence of a potentialization achieved by the inhibitory effect of losartan and dipyrone on COX enzymes. Furthermore, Gonçalves et al. [31] reported that the combination of losartan and nitroflurbiprofen, a NSAID, reduced vascular and glomerular COX-2 expression. On the other hand, Boshra et al. [32] studied the effect of combined use of a selective COX-2 inhibitor, celecoxib and losartan, on the mean arterial blood pressure (MAP) and plasma prostaglandin E_2 (PGE_2) levels in rats. They found that losartan decreased MAP by increasing PGE_2 levels whereas celecoxib did not alter the MAP lowering effect of losartan but decreased PGE_2 levels. This result seems to indicate that losartan is not associated with COX-2 inhibition. However, considering that dipyrone inhibits COX-3 + COX-1 at moderate and COX-3 + COX-1 + COX-2 at high concentrations while it inhibits COX-3 at low concentrations, we may suggest that losartan may modulate other COX isoforms, since we observed that moderate and high concentrations of dipyrone augmented the relaxant effect of losartan on Phe-induced contractions whereas low, moderate, and high concentrations of dipyrone made an enhancement on KCl-induced contractions, respectively.

It was reported that ACE-Is induced vasorelaxation by increasing bradykinin levels via NO-dependent pathway [33]. However, Yamada et al. [34] observed that indomethacin, a COX inhibitor, inhibited the vasorelaxation induced by the ACE-I rapakinin. They suggested that COX products, especially prostaglandines mediated the vasorelaxant effect of rapakinin. In contrast to losartan, it was found that ACE-I enalapril was not associated with COX-2 expression in rabbit hearts but increased the relaxing effects of endogenous prostaglandins on isolated mesenteric arteries and the researchers suggested that the contribution of ACE inhibition on these effects were independent from AT_1 receptors [17]. In an hypertension model in rats, it was reported that captopril, an ACE-I, suppressed the blood pressure raise, by lowering plasma AngII and increasing COX-2-derived 6-keto-PGF(1α) which is a prostacyclin PGI2 metabolite [35]. Accorsi-Mendonça et al. [28] studied the effects of the ACE-I, trandolapril with indomethacin on Phe- and AngII-induced contractions on isolated carotid arteries in rats. They suggested that indomethacin did not change the effect of trandolapril on the potency values of both agonists. In contrast, we observed that moderate and high concentrations of dipyrone augmented the relaxant effect of the ACE-I lisinopril on Phe-induced contractions whereas a potentiated effect was observed with low concentration of dipyrone and lisinopril combination on KCl-induced contractions. Thus, we may suggest that lisinopril modulates effect of dipyrone.

It is suggested that NSAIDs influence blood pressure and that the concomitant administration of NSAIDs may attenuate the blood pressure lowering effects of antihypertensive drugs. Some of the reports indicated that

NSAIDs/COX inhibitors increased blood pressure whereas some studies reported no change or lowering of blood pressure [32]. In our study, an NSAID drug dipyrone alone showed a vascular relaxation on precontracted aorta and augmented the vascular relaxing effects of losartan or lisinopril.

As a conclusion, we suggest that COX inhibition achieved with dipyrone may be responsible for the augmentation of the smooth-muscle relaxing effects of losartan or lisinopril. The inhibition of contractile prostanoids may be the contributing factor to the increase in the relaxation. Furthermore, dipyrone may be preferred if there is a need to use losartan or lisinopril with a NSAID.

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