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Alendronate Decreases Contractile Responses by Affecting ATP-Sensitive Potassium Channels in Human Left Internal Mammary Artery Rings

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Abstract: Bisphosphonates, including alendronate, are widely used for the treatment of osteoporosis, hypercalcemia and bone metastasis associated with cancer. However, recent evidence has demonstrated anti-atherosclerotic effects of bisphosphonates in animal models and atherosclerotic patients. Besides, some studies have shown that bisphosphonates change calcium homeostasis in cardiomyocytes and interfere L-type calcium channels in vascular smooth muscle cells. Therefore, in the present study it was aimed to investigate the effects of alendronate on contractile responses and ATP-sensitive potassium channels *in vitro* in human Left Internal Mammary Artery (LIMA). Human LIMA rings were placed into isolated organ chambers. After resting period, rings were kept for an hour in incubation medium which contains alendronate (10^{-6} M) or not (control) and responses of the rings to the contractile agents were examined. The incubation of two rings was performed in the presence of glibenclamide which is ATP-sensitive potassium channel blocker (10^{-6} M). Maximum contractile responses of LIMA rings to noradrenalin and serotonin decreased in the presence of alendronate. In concomitant incubation of LIMA with alendronate and glibenclamide reversed decreased contractions to noradrenalin or serotonin caused by alendronate. However, neither alendronate nor glibenclamide changed sensitivity to noradrenaline or serotonin. The present findings point out that alendronate may change intracellular calcium dynamics in human LIMA, due to the activation of ATP-sensitive potassium channels which have an important role on cardiovascular system.

Key words: Bisphosphonates, alendronate, LIMA, contraction, potassium channels

INTRODUCTION

Bisphosphonates are widely used in treatment and prevention of osteoporosis, treatment of bone metastases, multiple myeloma and Paget's disease (Drake *et al.*, 2008). They decrease osteoclast-mediated bone resorption and metastatic bone disease. In addition to their primary indication in clinical practice, their effects on various organ systems and diseases have been studied.

Potential beneficial effects of bisphosphonate treatment on cardiovascular system have also been investigated in both experimental and clinical studies (Santos *et al.*, 2012). Although, bisphosphonates are highly hydrophilic compounds, they can accumulate in the wall of atherosclerotic and healthy arteries where they may have useful effects (Santos *et al.*, 2012; Ylitalo *et al.*, 1996, 1998). When administered orally or intravenously,

after redistribution, small amounts of bisphosphonates remained in soft tissues are gradually released over a period of several days to weeks (Kemeny-Suss *et al.*, 2009).

Bisphosphonates can inhibit adhesion, proliferation and migration of vascular smooth muscle cells (Albadawi *et al.*, 2013; Wu *et al.*, 2009). They can also alter phenotype of vascular smooth muscle cells from synthetic to contractile type (Su *et al.*, 2002). Besides, it has been reported that etidronate prevents and inhibits progression of vascular calcification in hemodialysis patients (Hashiba *et al.*, 2004, 2006). Similarly, Zhou *et al.* (2013) proved that alendronate inhibited vascular calcification through down-regulation of the Notch1-RBP- $\text{J}\kappa$ signalling pathway. In the same study, it was highlighted that alendronate decreases high calcium content in vascular smooth muscle cells (Zhou *et al.*, 2013).

Accordingly, risedronate was shown to significantly suppress arterial wall stiffness in female osteoporosis patients (Okamoto *et al.*, 2010). Bisphosphonates were also introduced to improve elasticity of small and large arteries (Luckish *et al.*, 2008).

All of these actions of bisphosphonates may contribute to their beneficial effects on cardiovascular diseases. Indeed, their inhibitory effects on neointimal hyperplasia (intimal thickening) have been reported in previous studies (Santos *et al.*, 2012; Ylitalo-Heikkala, 2006). Clodronate inhibited the development of diet-induced atherosclerosis without influencing cholesterol levels in rabbits (Ylitalo *et al.*, 1994). Zoledronate also suppressed neointimal hyperplasia and gelatinase up-regulation in carotid artery anastomosis model in rabbits (Guzeloglu *et al.*, 2011). Alendronate treatment significantly decreased carotid artery intima-media thickness in women with postmenopausal osteoporosis (Celiloglu *et al.*, 2009).

Although, there is a growing body of evidence to support effective role of bisphosphonates in atherosclerosis, limited number of studies focused on the effects of bisphosphonates on contractile responses of vascular smooth muscle. Clodronate was reported to inhibit contraction in perfused rat-tail artery mediated by noradrenaline and depolarizing solution (Paspaliaris and Leaver, 1991). In a similar study, clodronate and pamidronate were demonstrated to reduce human arterial contractile response to alpha-adrenergic and depolarizing agents (Ylitalo *et al.*, 1998).

Human internal left mammary artery (LIMA) is routinely used as a graft in coronary artery bypass surgery which is considered as gold standard (Rosenfeldt *et al.*, 1999; Tector *et al.*, 1983). The LIMA spasms occur frequently during the surgical manipulation and also in postoperative period (Gojkovic-Bukarica *et al.*, 2011; Rosenfeldt *et al.*, 1999). Postoperative LIMA spasm which is characterised by ST-segment elevation in the grafted territory, rising cardiac enzymes, hemodynamic instability and cardiac arrest is a highly lethal condition (Tector *et al.*, 1983). Therefore, LIMA spasms limit its use. Attempts to overcome spasm can also lead to structural damage in the graft which may impair short and long-term patency (Rosenfeldt *et al.*, 1999). Numerous pharmacological agents have been studied for the prevention and treatment of graft spasm (Gojkovic-Bukarica *et al.*, 2011; Rosenfeldt *et al.*, 1999).

Under the light of this knowledge, the present study aimed to investigate the effects of alendronate, a nitrogen-containing bisphosphonate on adrenergic and

serotonergic contractions and to clarify the potential contribution of K_{ATP} channels to its effects on contractions in LIMA.

MATERIALS AND METHODS

Drugs: All drugs were dissolved in distilled water and diluted with 0.9% NaCl for cumulative concentrations. All drugs and reagents were purchased from Sigma, USA.

Selection of patients: The remaining segments of LIMA from male patients who had undergone coronary bypass surgery were used in organ chamber experiments. The mean age of the patients was 63 ± 3.66 . Since, diabetes, calcium channel blockers and nitrates may affect the results, diabetic patients and patients who had used calcium channel blocker and/or long-acting nitrates in 48 h before surgical intervention were excluded. Similarly, artery segments from the patients who suffered operative mortality or cardiac morbidity were also excluded from the experiments.

Approval: All patients gave their informed consent for excision of the remaining tissue. The experimental protocol was approved by the Ethical Committee of Izmir University (Protocol number is 2012/06). Research was carried out in accordance with Declaration of Helsinki of the World Medical Association.

Vascular preparations: Artery segments were put into cold (4°C) DMEM/F12 cell culture medium immediately and taken to the laboratory. Firstly, LIMA segments were dissected. Following careful removal of loose connective tissue, they were cut into 2 mm rings. The endothelial layers of the rings were removed mechanically by scratching in order to exclude the effects of endothelial derived relaxant and/or hyperpolarizing factors. Four rings from each artery segment were used. Rings were mounted between 2 stainless steel hooks in the organ chamber (PanLab, Spain) filled with 10 mL 37°C Krebs solution continuously gassed with 95% O_2 , 5% CO_2 . Krebs solution contained (mM): NaCl, 118, KCl, 4.7, CaCl_2 , 2.5, KH_2PO_4 , 1.2, MgSO_4 , 1.2; NaHCO_3 , 25 and glucose, 11.1. Contractile force changes were measured with an isometric force transducer (ADInstruments, USA) and recorded by a computer program (LabChart 7.0, ADInstruments, USA). The artery rings were gradually stretched to a tension of 2 g which was previously determined as optimal resting tension based on the length-tension relationship. Segments were then allowed

to equilibrate for 60 min at their optimal length. During the resting period, the Krebs solution in the organ chambers was changed after every 15 min.

Isolated organ chamber experiments: Artery rings were contracted with single dose of noradrenaline (3×10^{-5} M) at the end of resting period to be sure whether all the rings have equal contractile capacity. Rings were then kept for an hour in incubation medium which contains alendronate (10^{-6} M) or not (control). The incubation of two rings was performed in the presence of glibenclamide (10^{-6} M). Concentration-response relationships to cumulative concentrations of noradrenaline (10^{-9} - 10^{-4} M), serotonin (5-HT) (10^{-9} - 3×10^{-5} M) and nitroglycerin (10^{-9} - 3×10^{-5} M) were investigated in each preparation. Each agonist was washed out by changing the chamber solution three times within 30 min before addition of the next agonist.

Statistical analysis: All data was expressed as Mean \pm SEM. The $p \leq 0.05$ was considered statistically significant. Means were compared by paired Student's t-test. Values of maximal effect (E_{max}) and 50% effective concentration (EC_{50}) were derived for each cumulative concentration-response curve with iterative non-linear curve fitting (GraphPad Prism, 4.03, USA). The means of the negative logarithm of EC_{50} values (pD_2 values) were compared. All contraction responses were normalized with dry weight of the rings. Nitroglycerin-induced relaxations were normalized to single dose noradrenaline pre-contraction.

RESULTS

Effects of alendronate on noradrenaline-induced contractions: Alendronate incubation significantly decreased noradrenaline-induced maximum contractions in LIMA rings. Glibenclamide incubation did not change maximum contractile responses to noradrenaline. However, concomitant incubation of LIMA rings with alendronate and glibenclamide, normalized declined contractions to noradrenaline. Neither alendronate nor glibenclamide affected sensitivity to noradrenaline (Fig. 1, Table 1).

Effects of alendronate on 5HT-induced contractions: Alendronate incubation significantly reduced 5-HT-induced maximum contractile responses in LIMA rings. Glibenclamide incubation did not change contractile responses to 5-HT. Glibenclamide reversed decreased

Table 1: Effects of alendronate and glibenclamide on pD_2 values for noradrenaline or serotonin-induced contractions and nitroglycerin-induced relaxations

Control	Alendronate	Glibenclamide	Glibenclamide +Alendronate
Noradrenaline (n = 6) 6.942 \pm 0.217	6.997 \pm 0.263	7.063 \pm 0.170	7.130 \pm 0.106
5-HT (n = 6) 5.807 \pm 0.124	5.700 \pm 0.185	5.938 \pm 0.074	5.809 \pm 0.122
Nitroglycerin (n = 5) 6.975 \pm 0.205	7.223 \pm 0.150	7.088 \pm 0.200	6.677 \pm 0.072

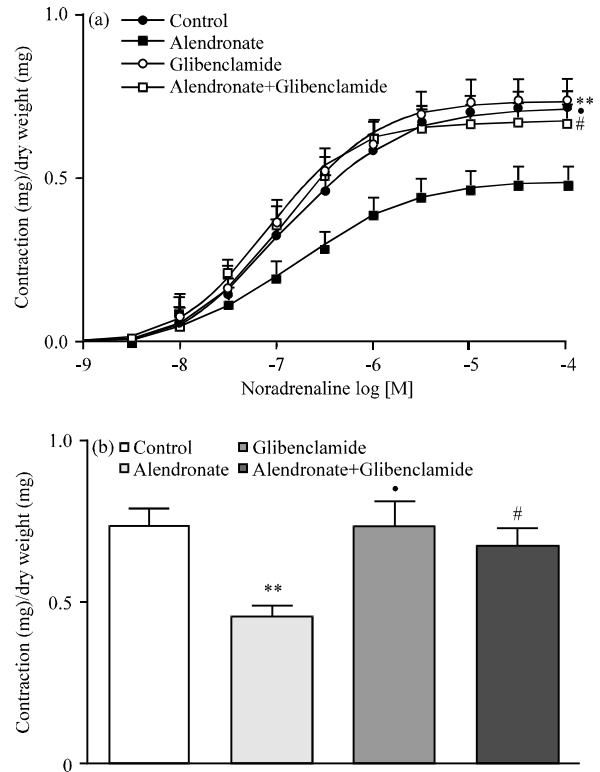


Fig. 1(a-b): (a) Effects of alendronate and glibenclamide on noradrenaline-induced contractions (n = 6) and (b) Maximum contractile response to the same drug, ** $p \leq 0.01$ (control vs. alendronate), • $p \leq 0.05$ (glibenclamide vs. alendronate), # $p \leq 0.05$ (alendronate vs. alendronate plus glibenclamide), paired Student's t-test

contractions to 5-HT caused by alendronate. Either alendronate or glibenclamide did not alter sensitivity to 5-HT (Fig. 2, Table 1).

Relaxation to nitroglycerin in noradrenaline pre-contracted LIMA rings: Neither alendronate nor glibenclamide affected relaxation responses or sensitivity to nitroglycerin in noradrenaline-precontracted LIMA rings (Fig. 3, Table 1).

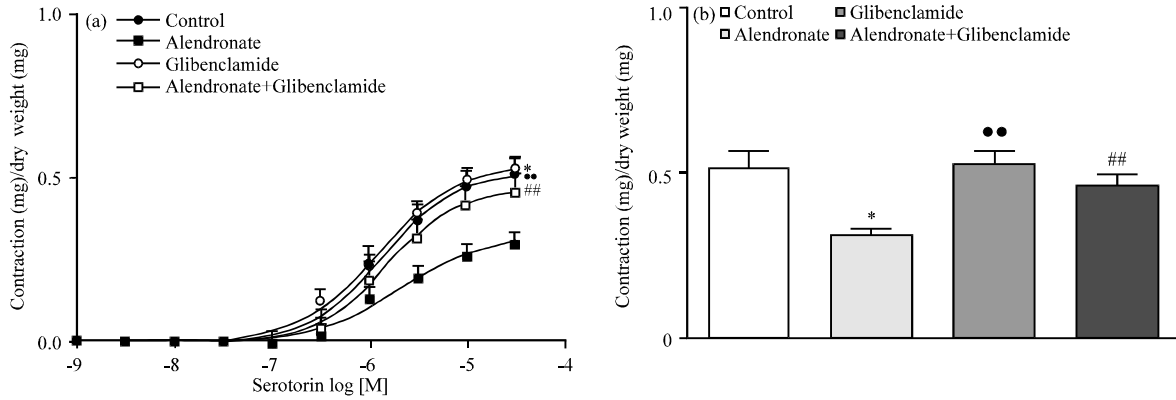


Fig. 2(a-b): (a) Effects of alendronate and glibenclamide on 5-HT-induced contractions (n = 6) and (b) maximum contractile response to the same drug, *p \leq 0.05 (control vs. alendronate), **p \leq 0.01 (glibenclamide vs. alendronate), ##p \leq 0.01 (alendronate vs. alendronate plus glibenclamide), paired Student's t-test

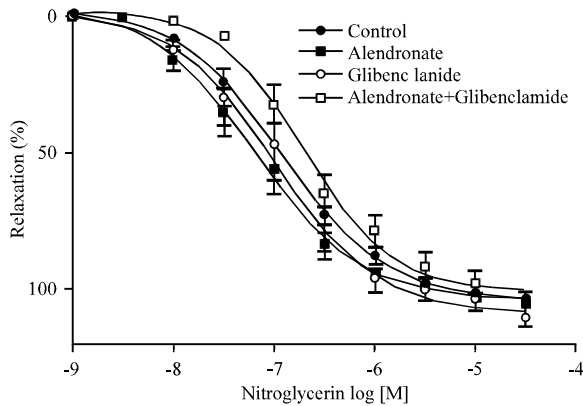


Fig. 3: Effects of alendronate and glibenclamide on nitroglycerine-induced relaxations in NA-precontracted LIMA rings (n = 5)

DISCUSSION

In the present study, it was demonstrated that alendronate decreased adrenergic and serotonergic receptor-dependent maximum contractions without changing sensitivity to these contractile agents in human left mammary artery. In this context, it was shown first time that alendronate inhibits contractile responses to serotonin. It was also revealed first time that inhibitory effects of bisphosphonates on adrenergic and serotonergic contractile responses at least partially may result from the effects of these agents on K_{ATP} channels.

In consistent with the present findings, Paspaliaris and Leaver (1991) reported that clodronate decreased vasoconstriction of isolated perfused rat tail arteries. They suggested that bisphosphonates can affect calcium efflux and clodronate has two type of action on vascular smooth muscle contraction; the first one on

intracellular mobilization of Ca^{+2} and the second on L-type Ca^{+2} channels. Similarly, Ylitalo *et al.* (1998) demonstrated that clodronate and pamidronate reduced human arterial contractile response to alpha adrenergic and depolarizing stimuli. Interestingly, they reported that these effects may not associated with L-type calcium channels and also pointed out that clodronate and pamidronate may reduce the contractions of human artery at the cell level without affecting intracellular Ca^{+2} metabolism.

The present evidence related with the decrement of vascular contractions due to alendronate are consistent with the data from the earlier studies on other bisphosphonates, clodronate and pamidronate. To our knowledge, previous studies with bisphosphonates focused on adrenergic and potassium chloride mediated contractions. In the present study, it was shown first time that bisphosphonates can also decrease serotonergic contractions.

In the present study, pD_2 values of adrenergic and serotonergic contractile agents did not change. This data suggest that inhibitory effects of bisphosphonates on contractile responses may independent from the mechanisms on receptor level.

Alendronate did not change relaxation response to nitroglycerin. Thus, it was indirectly demonstrated that the effect of alendronate on contractions is not related with relaxation mechanisms of vascular smooth muscle.

Effects of alendronate on calcium dynamics have been also reported in cardiomyocytes. Short and long term exposure to alendronate delayed caffeine-induced intracellular calcium responses and slowed the rate of calcium increase (Kemeny-Suss *et al.*, 2009).

K_{ATP} channels are metabolically found in many different mammalian cell types including human coronary smooth muscle (Farouque and Meredith, 2007). Opening

of K_{ATP} channels results in diffusion of potassium to the extracellular space and hyperpolarization of the smooth muscle cell membrane. The consequence of this change in membrane potential is closure of voltage-dependent calcium channels, thereby decreasing calcium influx (Farouque and Meredith, 2007).

It was hypothesized that bisphosphonates may change intracellular calcium concentration by affecting ATP sensitive potassium channels. In the present study, glibenclamide normalized decreased contractile responses caused by alendronate. Glibenclamide is a widely used antidiabetic drug, from the class of sulfonylureas. Glibenclamide was used as K_{ATP} channel inhibitor. About 1-10 μ M glibenclamide concentrations have been shown to inhibit K_{ATP} channels in human internal mammary artery (Gojkovic-Bukarica *et al.*, 2011).

Interestingly, it was reported that bisphosphonates may relieve of pain associated with inflammation in certain clinical situations (Varena, 2014). Accordingly, a previous study demonstrated that bisphosphonates (etidronate and alendronate) exhibit antiallodynic activity related with K_{ATP} channels in arthritic rats (Kawabata *et al.*, 2006). These results support the present findings and suggest that bisphosphonates may affect ATP-sensitive potassium channels in various tissues. However, further studies on short-term and long-term effects of bisphosphonates on ATP-sensitive potassium channels will broaden our knowledge.

As mentioned in introduction, LIMA spasms limit its use in coronary artery bypass surgery and intervention to LIMA spasms may lead to structural damage to the graft and may affect achievement rate of the coronary artery bypass graft (Rosenfeldt *et al.*, 1999; Tector *et al.*, 1983). Therefore, patients who undergone to coronary bypass surgery may have advantage to protection of graft patency if they use bisphosphonates for different indications eg, osteoporosis. In these patients, alendronate may be useful to increase the achievement rate of LIMA use and decrease the complications due to LIMA spasm. Further meta studies may contribute to reveal these possible useful effects of bisphosphonates.

CONCLUSION

In conclusion, the present results point out again to direct physiological effects of bisphosphonates on human LIMA. These effects seem to be related to ATP-sensitive potassium channels in vascular smooth muscle.

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