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Influence of Zoledronic Acid on Atrial Electrophysiological Parameters and Electrocardiographic Measurements

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Abstract

Introduction—Our objective was to determine effects of zoledronic acid (ZA) on atrial electrophysiological parameters and electrocardiographic measurements.

Methods and Results—*Ex vivo perfusion study:* Isolated guinea pig hearts were perfused with modified Krebs-Henseleit (K-H) buffer with or without ZA 0.07 mg/kg/L (each n=6). In ZA-perfused hearts, atrial action potential at 90% repolarization (APD_{90}) decreased more from baseline than in controls (−23.2% ± −5.1% vs −2.1% ± −8.1%, p<0.0001), as did APD_{30} (−28.8% ± −3.8% vs −2.1% ± −2.1%, p<0.0001). *In vivo dose-response study*: Guinea pigs underwent intraperitoneal injections every two weeks in one of 4 groups (each n=8): ZA 0.007 mg/kg (low-dose), ZA 0.07 mg/kg (medium-dose), ZA 0.7 mg/kg (high-dose) or placebo. Hearts were excised at 8 weeks and perfused with modified K-H. Atrial effective refractory period (ERP) was lower with medium and high-dose ZA versus placebo (p=0.004). Atrial APD_{30} was lower with high-dose ZA versus placebo, low and medium doses (p<0.001). *Canine ECG study*: Mature female beagles received intravenous ZA 0.067 mg/kg or saline (placebo) (each n=6) every two weeks for 12 weeks. P wave dispersion was greater in the ZA group (7.7±3.7 vs. 3.4±2.6 ms, p=0.04). There were no significant differences in P wave index, maximum or minimum P wave duration, or PR interval.

Conclusion—ZA shortens left atrial APD and ERP and increases P wave dispersion.

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Keywords

Action potential; atrial fibrillation; bisphosphonates; electrocardiography; PR interval; P wave; proarrhythmia; zoledronic acid

Introduction

Osteoporosis occurs in ~ 50% of women and ~ 20% of men over the age of 50,[1] and is associated with a high incidence of fractures. Bisphosphonate drugs reduce the risk of fractures in patients with osteoporosis,[2] and have become the gold standard for treatment. Bisphosphonates are also recommended for bone pain and fracture prevention in patients with metastatic cancer,[3] and are increasingly used in this patient population. In addition, bisphosphonate drugs are used for improvement in bone mineral density in patients with primary hyperparathyroidism,[4] reduction in bone turnover in patients with Paget's disease,[5] prevention and treatment of glucocorticoid-induced osteoporosis,[6] and hypercalcemia of malignancy.[7] Numerous bisphosphonates are available, including alendronate, etidronate, ibandronate, pamidronate, risedronate, tiludronate, and zoledronic acid. Approximately 30 million prescriptions are written for bisphosphonates annually in the United States.[8]

In 2007, a randomized, double-blind, placebo-controlled study in nearly 4,000 patients with osteoporosis found that zoledronic acid significantly increased the incidence of serious atrial fibrillation (AF) (1.3% versus 0.5%, $p<0.001$).[2] In addition, in a placebo-controlled study of 6459 women with osteoporosis, a trend towards an increased incidence of serious AF was reported in association with alendronate (1.5% versus 1.0%, $p=0.07$).[9] Since that time, a growing body of data has associated bisphosphonates with an increased risk of serious AF and overall AF. Data from case-controlled studies,[10] cohort studies[11] and meta-analyses[12] have also found significant associations between bisphosphonates and serious AF. In addition to these reports in patients receiving bisphosphonates for osteoporosis, studies have reported an association between bisphosphonates and AF, including serious AF, in patients with cancer receiving bisphosphonates for fracture prevention.[13,14] Overall, a total of 12 published studies have reported an association between bisphosphonates drugs and new onset and/or serious AF. However, conflicting data exist; some investigators have not found an association between bisphosphonates and AF;[15,16] a lack of association between bisphosphonates and new onset and/or serious AF has been reported in 12 published studies.

Identification of the potential for bisphosphonates as possible causes of new onset AF is clinically important. AF causes symptoms including palpitations, shortness of breath, and dizziness, which negatively influence quality of life. Further, AF is associated with a 5-fold increase in the risk of stroke, which can result in permanent paralysis or death, and a 2-fold increase in the risk of cardiovascular death. Thus, AF induced by a class of drugs as widely prescribed bisphosphonates may have serious public health consequences. However, as mentioned above, not all studies have found an association between bisphosphonate drugs and AF, and questions remain regarding the proarrhythmic potential of these agents.

In view of the disparate and conflicting data regarding the potential for bisphosphonates to provoke new-onset AF, we sought to test the hypotheses that zoledronic acid shortens atrial repolarization and influences P wave measures associated with an increased risk of AF.

Methods

This investigation consisted of three experiments: 1) *Ex vivo* zoledronic perfusion of isolated guinea pig hearts; 2) *In vivo* zoledronic dose response in guinea pigs, and 3) *In vivo* zoledronic administration to canines. This investigation conformed to the National Institute of Health's Guide for the Care and Use of Laboratory Animals, and was approved by the Institutional Animal Care and Use Committee at Indiana University Purdue University Indianapolis.

Ex vivo zoledronic acid perfusion

Female Dunkin-Hartley retired breeder guinea pigs (approximately 2.5 years old; n=12) were anesthetized with pentobarbital 50 mg/kg and anticoagulated with heparin 500 IU administered intraperitoneally. Additional doses of sodium pentobarbital 10 mg/kg were administered as needed. The diaphragm was accessed and the thorax opened with a bilateral incision, exposing the heart, which was rapidly excised and quickly mounted on a Langendorff isolated perfused heart apparatus (IH-SR 5, Harvard Apparatus, Inc., Holliston, MA, USA), and immersed in a tissue bath filled with modified Krebs-Henseleit buffer, which consisted of: NaCL 118.5mM, KCl 4.7mM, $MgSO_4$ 1.2mM, $NaHCO_3$ 25.0mM, KH_2PO_4 1.2mM, $CaCl_2$ 1.8mM, and glucose 11.0mM. All reagents were obtained from Sigma Chemical Co. (St. Louis, MO, USA). The perfusate was equilibrated to 37° ± 0.5° C using a temperature control system (Thermostatic Circulator E103, Harvard Apparatus) and infused with 95% O_2 & 5% CO_2 at pH 7.4. The perfusate was maintained at constant temperature (37° C) and aortic pressure (60 mm Hg) throughout the study. A bipolar pacing electrode was placed on the right atrium. Hearts were paced at a basic cycle length of 250ms (240 bpm) at twice the diastolic pacing threshold using an HSE Stimulator PSM Type 676 programmable stimulator (Hugo Sachs Elektronik, March-Hugstetten, Germany). One spring-loaded silver-silver chloride monophasic action potential (MAP) electrode was positioned on the left atrial epicardium. Following dissection and instrumentation, hearts were allowed to equilibrate for 10 minutes prior to initiation of the experimental protocol. Electrophysiological signals were amplified using a PLUGSYS module (MAP Module Type 703, Hugo Sachs Elektronik) that allows for the simultaneous recording of up to 4 MAP signals.

Hearts were randomized to perfusion with zoledronic acid 0.07 mg/kg/L dissolved in Krebs-Henseleit buffer (n=6) or Krebs-Henseleit buffer without zoledronic acid (control; n=6). After the 10-minute equilibration period, left atrial MAPs were recorded continuously for 20 minutes. Data were recorded and acquired using the HSE-IsoHeart W data acquisition system (Hugo Sachs Elektronik-Harvard Apparatus, March-Hugstetten). Three beats from each set of recordings were selected for analysis and averaged for measurement of action potential duration (APD) prior to initiation of zoledronic acid perfusion and at baseline in the control group and every 5 minutes for 60 minutes following baseline measurements.

APDs were measured at 90% (APD_{90}) and 30% repolarization (APD_{30}) by an investigator (JET) who was blinded to the assigned treatment.

In vivo chronic zoledronic acid administration in guinea pigs

Female Dunkin-Hartley guinea pigs (n=32) were randomized to receive intraperitoneal injections every 2 weeks for 8 weeks in one of the following groups (n=8 each):

- Zoledronic acid 0.007 mg/kg (low dose)
- Zoledronic acid 0.07 mg/kg (medium dose; consistent, on a mg/kg basis, with the dose used in patients with cancer[17])
- Zoledronic acid 0.7 mg/kg (high dose)
- Placebo (saline)

After 8 weeks, on the day after the last dose of zoledronic acid or placebo was administered, guinea pigs were anesthetized with pentobarbital 50 mg/kg and anticoagulated with heparin 500 IU administered intraperitoneally. Excised hearts were mounted on a Langendorff isolated perfused heart apparatus as described above. Hearts were paced at a basic cycle length of 250ms (240 bpm) at twice the diastolic pacing threshold using an HSE Stimulator PSM Type 676 programmable stimulator (Hugo Sachs Elektronik, March-Hugstetten, Germany). One spring-loaded silver-silver chloride monophasic action potential (MAP) electrode was positioned on the left atrial epicardium. Following dissection and instrumentation, hearts were allowed to equilibrate for 10 minutes prior to initiation of the experimental protocol. Electrophysiological signals were amplified using a PLUGSYS module (MAP Module Type 703, Hugo Sachs Elektronik) that allows for the simultaneous recording of up to 4 MAP signals.

Following the 10-minute equilibration period, left atrial effective refractory period (ERP), was determined three times, 1 minute apart, in the following manner: Trains of 8 paced beats (S_1) at a constant pacing cycle length (250 ms) were delivered, after which an extrastimulus (S_2) was delivered starting at 100 ms following the last beat of the train, and decreased at 5 ms decrements until the extrastimulus failed to generate an atrial depolarization. The longest S_1–S_2 interval that failed to generate a depolarization was defined as the ERP. APD_{90} and APD_{30} were determined by an investigator (JET) who was blinded to the assigned treatment and averaged from three consecutive beats at three times separated by one minute.

In vivo zoledronic acid administration to canines

Mature female beagle dogs (n=12) between 1–2 years of age (Marshall Farms, North Rose, NY) were randomized to receive intravenous zoledronic acid 0.067 mg/kg (n=6) every two weeks for 12 weeks or to an untreated control group (n=6);[18] this dose is consistent with doses administered to patients for prevention of fractures associated with metastatic cancer. To perform drug administration, animals were sedated with medetomidine 0.15 mg, which was reversed with atipamezole 1 mg at the end of the infusion. To administer zoledronic acid, an over-the-needle catheter was inserted into the cephalic or saphenous vein; the site of injection was alternated for each dose. Zoledronic acid was administered in 40 mL normal

saline and infused over 15 minutes, consistent with the administration of the drug in clinical practice.

After 12-weeks of treatment, dogs were sedated with propofol 8 mg/kg. Three 12-lead electrocardiograms (paper speed 25 mm/s), separated by one minute, were obtained for analysis of P wave measurements known to be associated with risk of AF.[19] P wave onset was determined as a positive deflection after the T wave preceding the QRS complex and deviating from the isoelectric line from the T wave to the P wave. The end of the P wave was defined as the return to isoelectric baseline immediately before the QRS complex.[20] Up to 5 consecutive P waves were measured in each of the 12-leads. Leads in which P wave onset or termination could not be clearly discerned were not included for measurement. All P wave measurements were performed by the same investigator (HJ), who was blinded to the assigned treatments.

Maximum P wave duration was defined as the longest P wave measured from any of the 12 ECG leads. P wave dispersion was defined as the difference between the maximum and minimum P wave duration across all 12 leads.[20,21] P wave index was defined as the standard deviation of P wave duration across all 12 leads.[19] Since in some studies bisphosphonates have not been shown to increase the incidence of overall AF, but rather only “serious” AF, we also measured PR interval to assess the possibility that bisphosphonates might have the potential to increase ventricular rates in AF by accelerating AV node conduction.

QRS duration and QT intervals were measured manually from lead II by an investigator (HJ) who was blinded to the assigned treatments. QT intervals were measured from the earliest QRS deflection to the end of the T wave, defined as the intersection of a tangent to the steepest slope of the last limb of the T wave and the baseline. QT intervals were corrected for heart rate using both the Bazett’s and Fridericia methods.

Statistical analysis

Analyses were performed using SPSS 17.0 (SPSS, Inc, Chicago, IL). Normality of continuous data was determined using the Kolmogorov-Smirnov test. *Ex vivo perfusion study:* % change in APD_{90} and APD_{30} from baseline over time was compared between the two groups using two-way repeated measures analysis of variance (ANOVA). *In vivo dose response study:* Mean ERP, APD_{90} and APD_{30} were compared across the four groups using ANOVA. Tukey’s Honest Significant Difference test was used *post-hoc* to analyze significant differences between the groups. *In vivo canine ECG study*: P wave indices in the two groups were compared using Student’s unpaired t-test. For all analyses, $p< 0.05$ was considered significant.

Results

Ex vivo zoledronic acid perfusion

The guinea pigs in the zoledronic acid and control groups were similar with respect to weight (978±47 vs 967±53g, p=0.71). The effect of acute zoledronic acid perfusion in isolated perfused guinea pig hearts is shown in Figure 1. Zoledronic acid perfusion resulted in a significant decrease in APD_{90} compared with hearts in the control group (Figure 1A).

The maximum decrease from baseline in APD_{90} in the zoledronic acid group was −23.2% ± −5.1%, compared with −2.1% ± −8.1% in the control group (Figure 2A, p<0.0001). Similarly, zoledronic acid perfusion resulted in a significant decrease in APD_{30} compared with hearts in the control group (Figure 1B). The maximum decrease from baseline in APD_{30} in the zoledronic acid group was −28.8% ± − 3.8% compared with − 2.1% ± −2.1% in the control group (Figure 2B, p<0.0001). There were no episodes of spontaneous AF.

In vivo zoledronic acid dose-response in guinea pigs

There was no significant difference in age of the guinea pigs between the low, medium, high dose and placebo groups (16.5±0.9, 16.0±1.4, 16.4±1.6, and 15.8±1.2 weeks, p=0.64). Similarly, there was no significant difference in weight of the guinea pigs between the low, medium, high dose and placebo groups (670±32, 650±39, 662±37, and 658±25g, p=0.34).

Dose-response effects of zoledronic acid on atrial ERP in isolated guinea pigs hearts are presented in Figure 3. Zoledronic acid provoked a significantly lower mean ERP compared with placebo across the dosing groups; *post-hoc* testing revealed significant differences between medium dose (p=0.004) and placebo and between high dose and placebo (p=0.02).

The effects of zoledronic acid administration on atrial APD_{30} are presented in Figure 4. Zoledronic acid elicited a significantly lower APD_{30} compared with control across the dosing groups. *Post-hoc* analysis revealed the following significant differences: high dose versus placebo (p=0.005); high dose versus low dose (p=0.0001); high dose versus medium dose (p=0.02). Zoledronic acid administration did not exert a significant effect on atrial APD_{90} (Figure 5). There were no episodes of spontaneous AF.

In vivo zoledronic acid administration in canines

The effects of zoledronic acid on ECG measures of atrial activity in beagle dogs are presented in Table 1. Mean P wave dispersion was significantly greater in the zoledronic acid group compared with that in the control group. There were no significant differences between the zoledronic acid and control groups in mean P wave index, maximum P wave interval duration, mean minimum P wave interval duration, or in mean PR interval. There were no significant differences between the zoledronic acid and control groups in mean QRS duration, Bazett's- or Fridericia-corrected QT intervals, or heart rate (Table 1). There were no premature atrial or ventricular depolarizations on any of the ECGs, nor were there any episodes of spontaneous AF.

Discussion

Zoledronic acid influenced electrophysiological and electrocardiographic indices associated with an increased risk of AF. Acute zoledronic acid perfusion in isolated, perfused guinea pig hearts resulted in significant decreases in left atrial APD_{90} and APD_{30}. Chronic intraperitoneal administration of zoledronic acid to guinea pigs significantly decreased left atrial ERP at medium and high doses, and significantly decreased left atrial APD_{30} at high doses. In addition, chronic intravenous administration of zoledronic acid to beagle canines resulted in a significant increase in P wave dispersion.

Numerous clinical studies have implicated bisphosphonates as inducers of new-onset AF.[2,9–14] In some reports, bisphosphonates were not associated with an overall increase in the incidence of new-onset AF, but were associated with an increased incidence of "serious" AF;[2,9,12] definitions of "serious" AF were not provided. However, other investigators have associated bisphosphonates with contributing to an increase in the risk of all new-onset AF, not only "serious" AF.[10–13] In contrast, some investigators have not found an association between bisphosphonate use and AF.[14,15] Reasons for this disparity in findings are unclear, although the studies differ in design, bisphosphonate drug(s) investigated, patient inclusion, and data analysis. The influence of bisphosphonates on atrial electrophysiology or electrocardiographic measurements has not been described previously. The current results represent novel experimental data showing that zoledronic acid exerts electrophysiological and ECG effects known to be associated with an increased risk of AF.

The basis for our study was the fact that there is a substantial number of published clinical trials, case-controlled studies, cohort studies, and meta-analyses that report that bisphosphonates use is associated with an increased risk of AF. The potential association of bisphosphonates with new onset AF is controversial, and an equal number of published studies have not reported an association between bisphosphonates and new-onset AF. Nonetheless, the growing number of published studies that have found an association between bisphosphonate drugs and the occurrence of new onset AF led us to hypothesize that bisphosphonate drugs may affect atrial electrophysiological and electrocardiographic measures that are known to be associated with an increased risk of AF.

Reduced atrial ERP and shortening of the atrial APD are features associated with AF, and conditions that lead to decreased atrial APD and ERP are associated with an increased risk of AF.[22] In the present study, acute zoledronic acid perfusion in isolated, perfused guinea pig hearts resulted in significant decreases in left atrial APD_{90} and APD_{30}. Chronic administration of zoledronic acid to guinea pigs led to a significant decrease in atrial ERP at medium and high doses, and a significant decrease in APD_{30} in the high dose group. Chronic zoledronic acid administration to guinea pigs resulted in a trend towards a significant decrease in APD_{90} ($p=0.08$). Reasons for the greater change in APD_{30} (20.3% decrease at highest dose) compared with APD_{90} (14.4% decrease at highest dose) are not clear, but it is possible that bisphosphonates alter early-phase atrial repolarization to a greater degree than later phase. Zoledronic acid administration also resulted in significant reduction in atrial ERP at both the medium and higher doses, but only reduced atrial APD_{30} at the highest dose. ERP most closely approximates APD_{70}, rather than APD_{90};[23] the fact that zoledronic acid significantly shortened ERP but not APD_{90} could also be due to a greater effect of zoledronic acid on early-phase atrial repolarization than later phase.

Mechanisms by which zoledronic acid decreases atrial ERP, APD_{30} and APD_{90} and increases P wave dispersion have not been studied. Bisphosphonates have been reported to provoke an inflammatory response,[24] which may contribute to the development of AF. Bisphosphonates cause acute kidney injury in some patients,[25] which could increase fluid volume and left atrial strain, resulting in AF. Although these mechanisms are possible, our *ex vivo* perfusion experiment suggests that zoledronic acid may exert direct atrial electrophysiological effects. Alendronate has been shown to induce oscillations in

intracellular calcium concentrations in atrial cells.[26] In our investigation, zoledronic acid affected early phase atrial repolarization to a great degree than later phase, which could suggest an effect on I_{kur} and/or calcium current.[27] The contribution of these and other potential mechanisms of bisphosphonates to the development of AF requires additional study. Future research should also investigate the propensity for bisphosphonates to induce AF in experimental models, and to determine whether there are differences among the available bisphosphonate drugs in their potential to induce AF and atrial electrophysiological changes that promote AF. In particular, in light of our findings indicating that direct perfusion of zoledronic acid to isolated guinea pig hearts alters atrial action potential duration, studies in single atrial myocytes to determine effects of bisphosphonates on specific ionic currents are desired.

The medium zoledronic acid dose selected for use in the *in vivo* guinea pig experiment and the zoledronic acid dose selected for use in the chronic *in vivo* canine experiment were similar, on a mg/kg basis, to the 4 mg dose recommended for patients for the management of hypercalcemia of malignancy, multiple myeloma and bone metastasis from solid tumors. The low and high zoledronic acid doses in the *in vivo* guinea pig experiment were 10-fold lower and higher, respectively. The zoledronic acid concentration perfused through the isolated guinea pig hearts was also selected to mimic the dose use for the above indications.

Specific P wave indices have been shown to be associated with an increased risk of AF. Maximum and minimum P wave duration have been shown to predict the development in AF in some studies.[19,20] However, associations between P wave duration and AF have been inconsistent, and other studies have not found maximum or minimum P wave duration to be independent predictors of the development of AF,[28] or have found P wave dispersion to be a better predictor of AF than P wave duration based on comparison of receiver operating characteristics curves.[29] In this investigation, zoledronic did not significantly influence maximum or minimum P wave duration.

In comparison with P wave duration, P wave dispersion has more consistently been shown to be associated with development or perpetuation of AF. Increased P wave dispersion was shown to be independently associated with an increased risk of new onset AF in broad populations of patients,[19] as well as in those with hypertension[28] and chronic obstructive pulmonary disease.[30] Increased P wave dispersion (but not maximum P wave duration) was independently associated with progression from paroxysmal to persistent AF.[20] Increased P wave dispersion was also associated with recurrence of AF after conversion to sinus rhythm.[21] Increases in postoperative P wave dispersion independently predict AF following coronary artery bypass graft (CABG) surgery.[31] Finally, a reduction in P wave dispersion following cardiac resynchronization therapy was associated in a lower incidence of new-onset AF in patients with heart failure.[32] Overall, a large body of evidence suggests that P wave dispersion may promote the development and/or perpetuation of AF.

P wave index, defined as the standard deviation of P wave duration across all leads, has also been shown to be a strong predictor of the development of AF.[19] Zoledronic acid increased P wave index by > 2-fold in the canines studied in this investigation ($p=0.06$). Zoledronic acid did not significantly influence PR interval, suggesting that these drugs may not affect

AV nodal conduction. Zoledronic acid had no significant effects on canine QRS duration, rate-corrected QT interval, or heart rate. Bonilla et al[33] also reported no effects of a bisphosphonate (ibandronate) on QT interval up to 4 weeks post-dosing, compared to baseline values, in canines.

Limitations of these investigations warrant consideration. In the *in vivo* guinea pig experiment, zoledronic acid was administered via the intraperitoneal route. While zoledronic acid has been administered intraperitoneally by other investigators to other small animal species,[34] the bioavailability of intraperitoneal zoledronic acid in those models and in the guinea pig is unknown. Therefore, while the medium zoledronic acid dose was selected to simulate therapeutic zoledronic acid doses administered to patients with cancer, it is unknown whether plasma zoledronic acid concentrations were similar to those achieved by the same mg/kg dose when administered intravenously to patients. The anesthetic agent used in the *in vivo* canine experiment was propofol, which has been shown to decrease P wave dispersion in patients receiving propofol anesthesia for surgery.[35] However, we employed a control group in addition to the experimental group; both groups received propofol anesthesia at the same dose. In contrast to the *in vivo* guinea pig experiments, in which both atrial ERP and APD were measured, only atrial APD (but not ERP) was measured in the *ex vivo* guinea pig experiments. This was because the *ex vivo* guinea pig experiments were conducted first, to test the initial hypothesis that acute perfusion of zoledronic acid shortens atrial repolarization, and to determine whether the data would support further experimentation regarding the influence of zoledronic acid on atrial electrophysiology.

Conclusions

In vivo zoledronic acid administration in guinea pigs significantly reduces left atrial ERP at medium and high doses, and reduces left atrial APD_{30} at high doses. Chronic intravenous zoledronic acid therapy in canines significantly increases P wave dispersion. Direct *ex vivo* zoledronic acid perfusion significantly decreases left atrial APD_{90} and APD_{30} in isolated guinea pig hearts, suggesting that zoledronic acid may exert direct effects on atrial electrophysiology.

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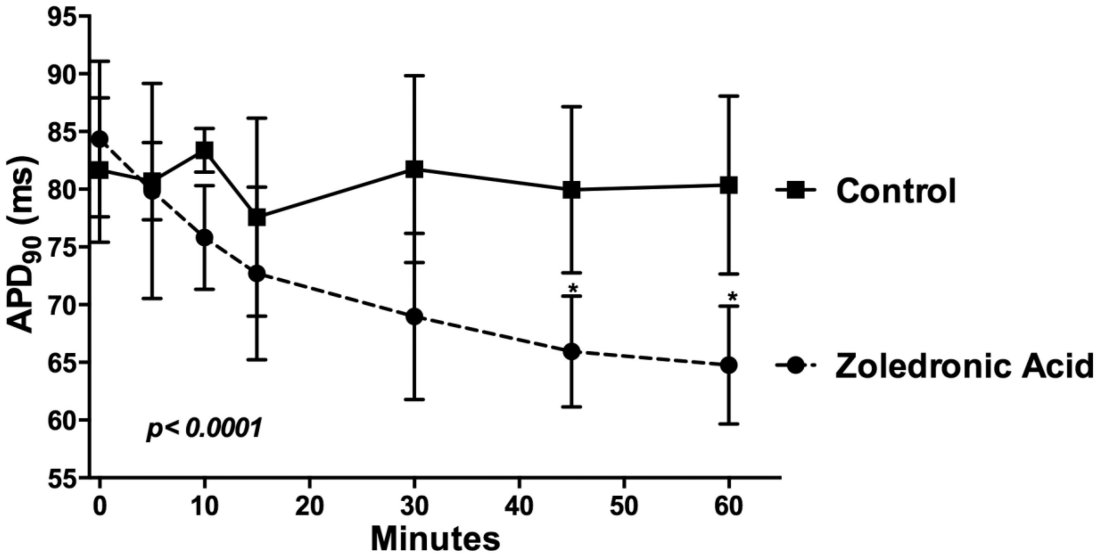
A.



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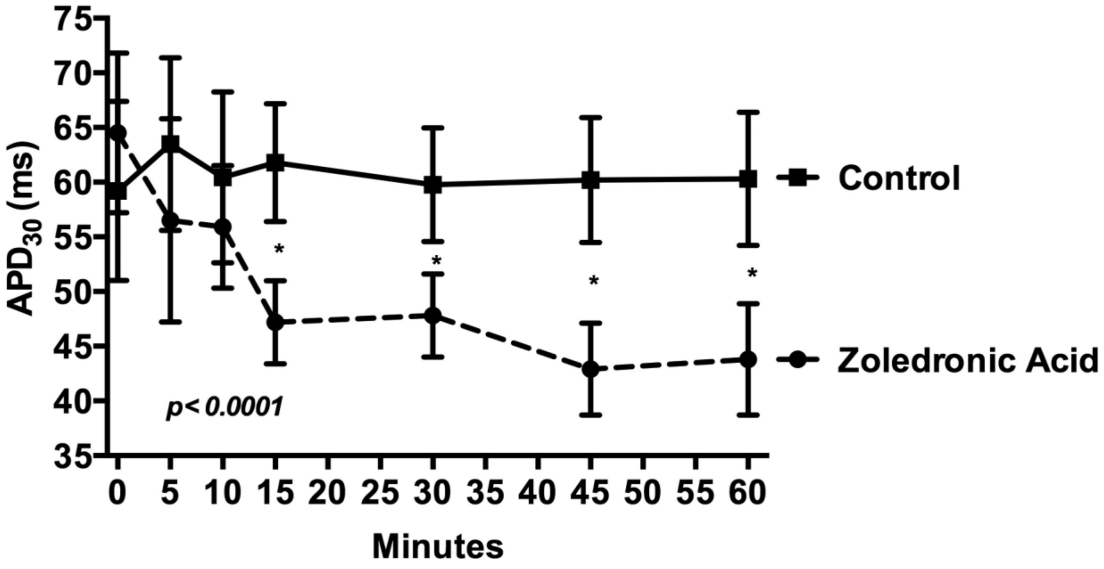

Figure 1.

Effects of perfusion of zoledronic acid on APD_{90} and APD_{30} in isolated perfused guinea pig hearts. Data presented as mean ± standard deviation

A. Effect on APD_{90}

B. Effect on APD_{30}

*p<0.05 compared to control at these time points

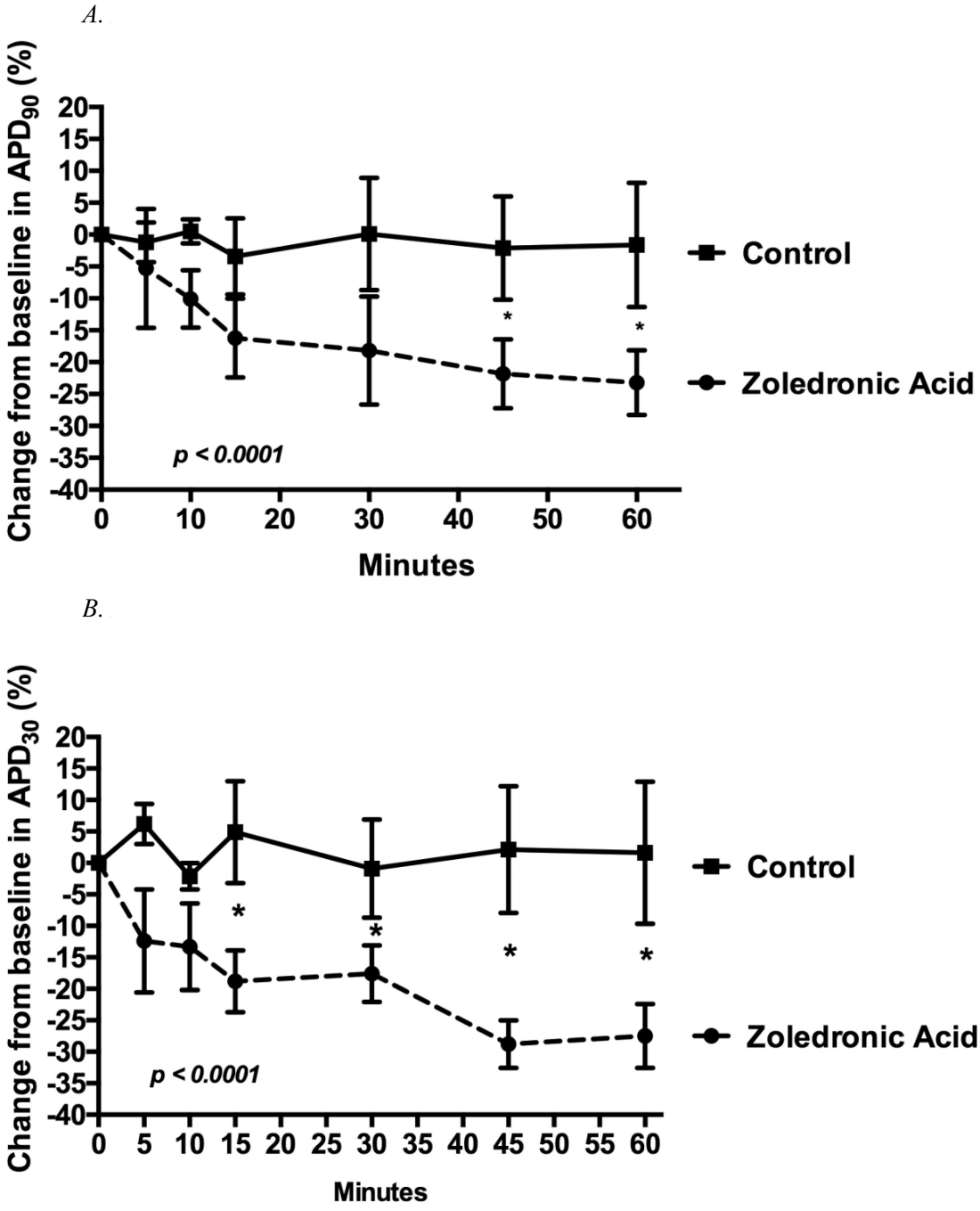

Figure 2.

Effects of perfusion of zoledronic acid on % change from baseline APD_{90} and APD_{30} in isolated perfused guinea pig hearts. Data presented as mean ± standard deviation

A. Effect on APD_{90}

B. Effect on APD_{30}

*p<0.05 compared to control at these time points

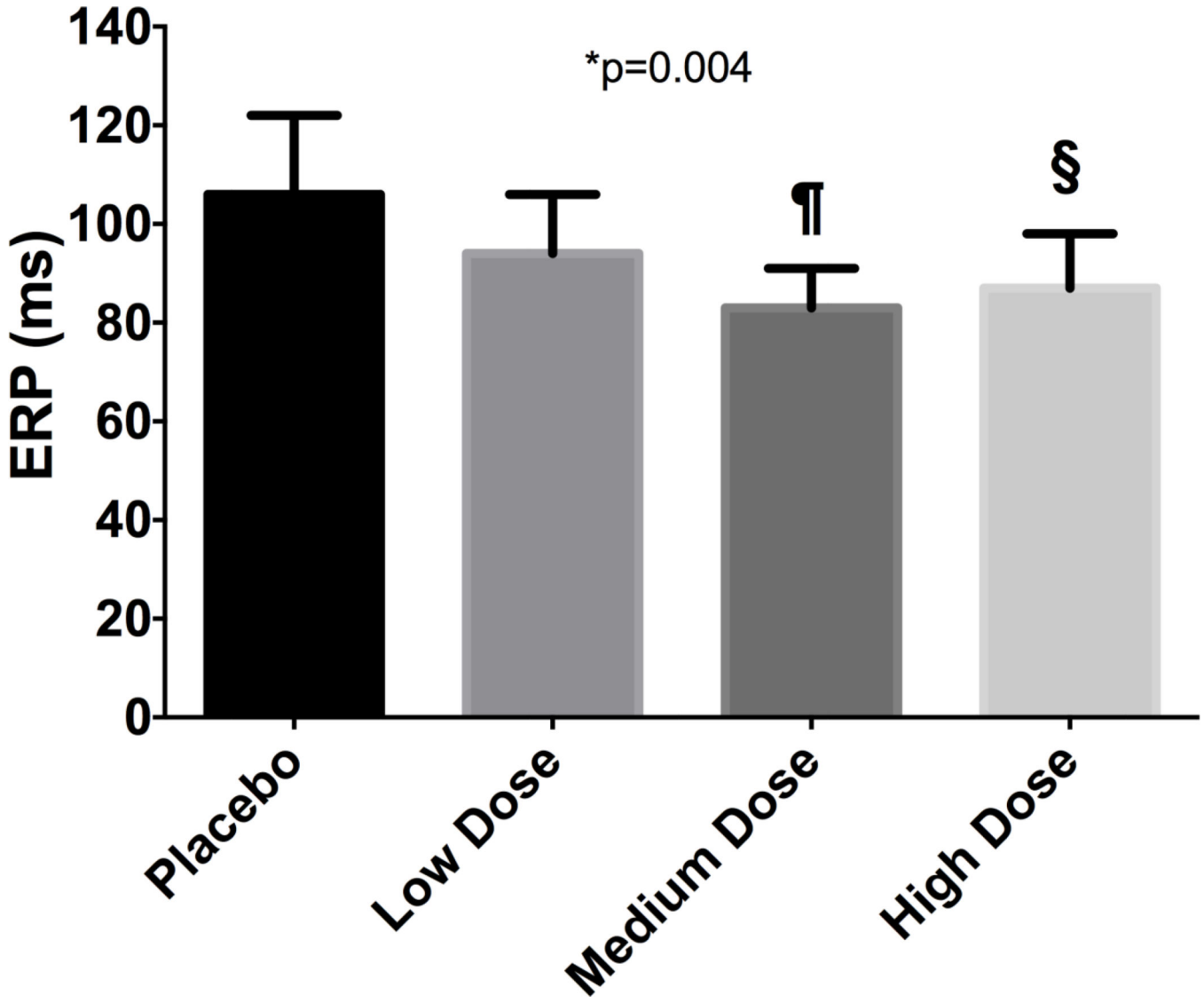

Figure 3.

Effects of chronic in vivo zoledronic acid administration on atrial effective refractory period (ERP) in guinea pig hearts. Data presented as mean ± standard deviation

*Analysis of variance p value comparing the four groups

¶Tukey's posthoc p=0.004 Medium dose compared to placebo

§Tukey's posthoc p=0.02 High dose compared to placebo

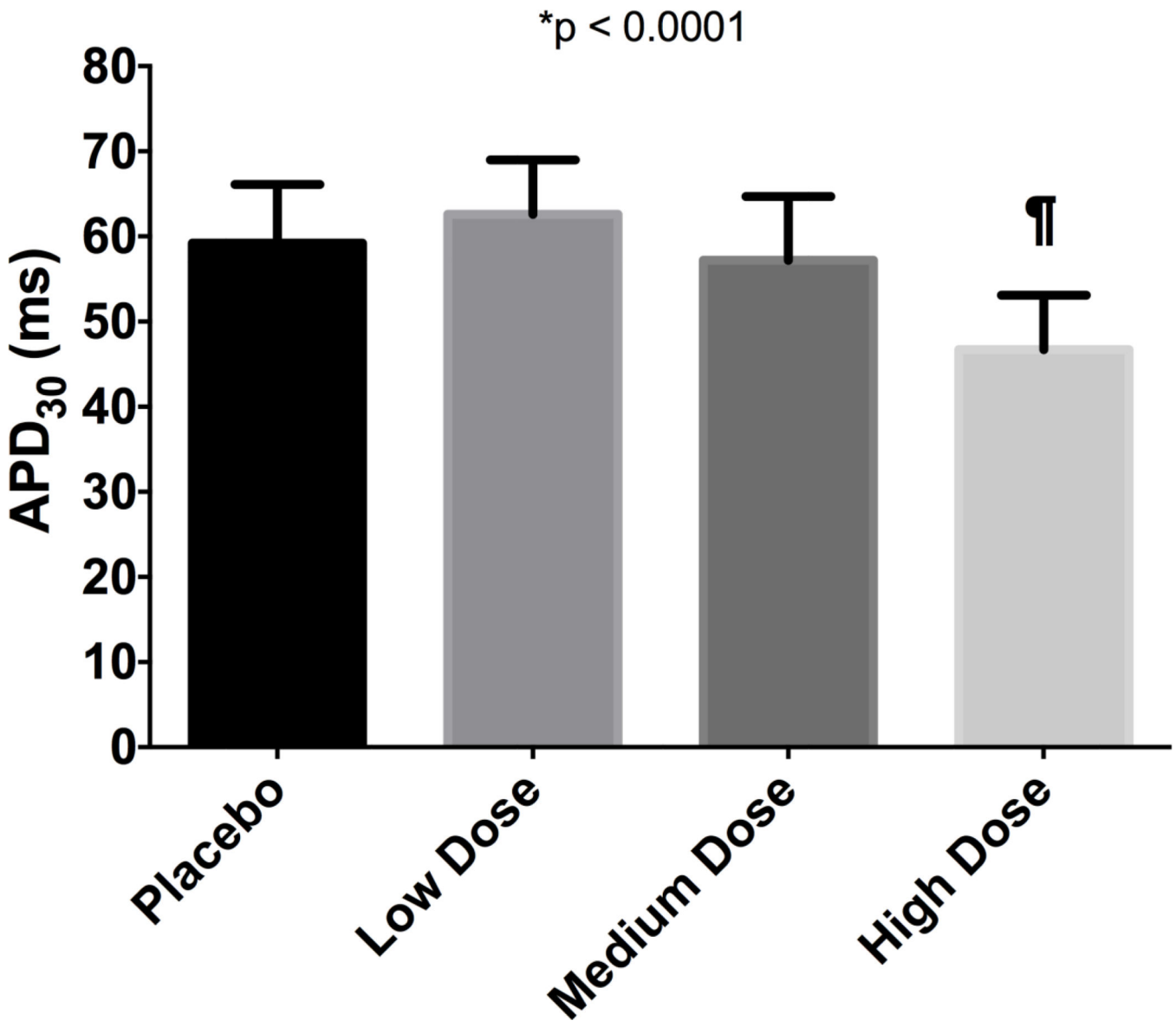

Figure 4.

Effects of chronic in vivo zoledronic acid administration on atrial action potential duration at 30% repolarization (APD_{30}). Data presented as mean ± standard deviation.

*Analysis of variance p value comparing the four groups

¶Tukey's posthoc p=0.005 high dose compared to placebo

¶Tukey's posthoc p<0.0001 high dose compared to low dose

¶Tukey's posthoc p=0.02 high dose compared to medium dose

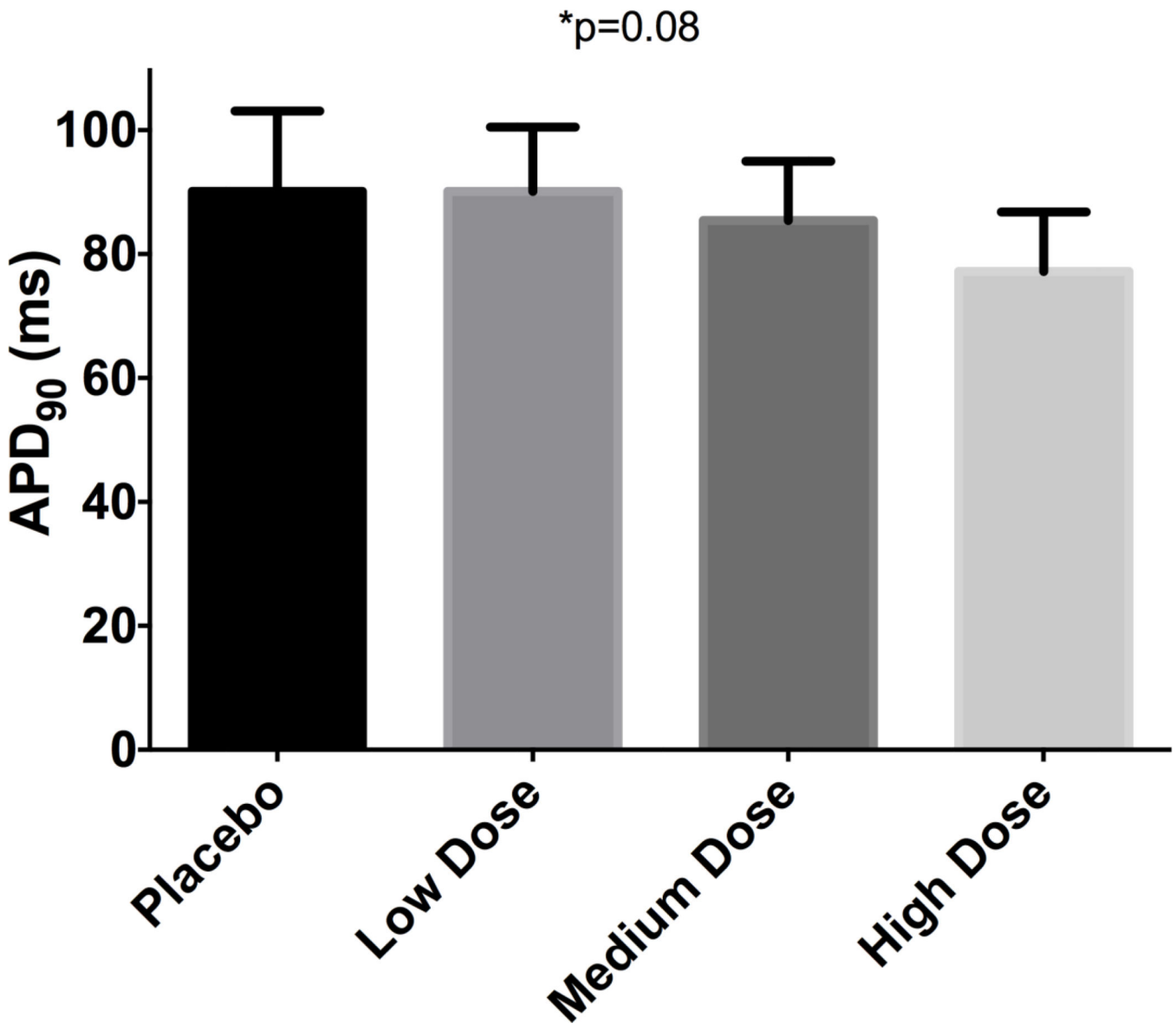

Figure 5.

Effects of chronic in vivo zoledronic acid administration on atrial action potential duration at 90% repolarization (APD_{90}). Data presented as mean ± standard deviation

*Analysis of variance p value comparing the four groups

Table 1

Effect of zoledronic acid on P wave measurements associated with increased risk of atrial fibrillation in dogs. Data presented as mean ± standard deviation.

	Zoledronic acid	Control	p
P wave dispersion (ms)	8 ± 4	3 ± 3	0.04
P wave index (ms)	42 ± 20	20 ± 15	0.06
Maximum P wave duration (ms)	39 ± 6	39 ± 11	0.97
Minimum P wave duration (ms)	31 ± 4	36 ± 9	0.29
PR interval (ms)	90 ± 16	91 ± 14	0.97
QRS duration (ms)	74 ± 10	67 ± 11	0.15
QT_c* interval (ms)	462 ± 22	460 ± 20	0.77
QT_F† interval (ms)	457 ± 18	454 ± 16	>0.99
RR interval (ms)	403 ± 44	405 ± 52	0.90
Heart rate (bpm)	151 ± 17	148 ± 19	0.64

*QT_C interval = Bazett's-corrected

†QT_F interval = Fridericia-corrected