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SHORT COMMUNICATION

Liraglutide suppresses atrial electrophysiological changes

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Abstract
We have shown that a dipeptidyl peptidase 4 (DPP-4) inhibitor suppresses atrial remodeling in a canine atrial fibrillation (AF) model. Glucagon-like peptide-1 (GLP-1) is increased by DPP-4 inhibitors. However, it is not clear whether GLP-1 is involved in the suppression of atrial remodeling. In this study, we evaluated the effect of liraglutide (a GLP-1 analog) on atrial electrophysiological changes using the same canine AF model. We established a canine AF model using continuous 3-week rapid atrial stimulation in seven beagle dogs divided into two groups: a liraglutide group with four dogs (3-week atrial pacing with liraglutide (150 μg/kg/day) administration) and a pacing control group with three dogs (3-week pacing without any medicine). We evaluated the atrial effective refractory period (AERP), conduction velocity (CV), and AF inducibility every week during the protocol using implanted epicardial wires against the surfaces of both atria. In the pacing control group, the AERP was gradually shortened and the CV was decreased along the time course. In the liraglutide group, the AERP was similarly shortened as in the pacing control group (94 ± 4% versus 85 ± 2%, respectively; $p = 0.5926$), but the CV became significantly higher than that in the pacing control group after 2 and 3 weeks (95 ± 4 versus 83 ± 5%, respectively; $p = 0.0339$). The AF inducibility was gradually increased in the pacing control group, but it was suppressed in the liraglutide group (5 ± 9% versus 73 ± 5%; $p = 0.0262$). Liraglutide suppressed electrophysiological changes such as AF inducibility and CV decrease in our canine AF model.

Keywords GLP-1 · Atrial fibrillation · Remodeling

Introduction

Atrial fibrillation (AF) is associated with atrial arrhythmogenic substrates that form due to electrical and structural remodeling [1]. We have documented the anti-remodeling effects of linagliptin, a dipeptidyl peptidase 4 (DPP-4) inhibitor, in a canine AF model [2]. Although the precise mechanism of the anti-remodeling effect of linagliptin remains unclear, we speculated that anti-oxidative and/or anti-inflammatory effects play a role. Because glucagon-like peptide-1(GLP-1) is increased after DPP-4 inhibitor administration [3–5], we hypothesized that GLP-1 could suppress the formation of AF substrates. In this study, we have evaluated the effects of liraglutide, a GLP-1 analog on the atrial electrophysiological changes in the canine AF model.

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Materials and methods

Animal model and initial surgery

We performed the initial surgery to establish the canine AF model as published [2, 6, 7]. Briefly, we anesthetized seven adult female beagle dogs (12.6 ± 1.2 kg) with pentobarbital (25–30 mg/kg IV) and administered butorphanol (0.3 mg/kg) for analgesia. We sutured bipolar epicardial leads at the surface of the right atrial (RA) and left atrial (LA) free walls, subcutaneously tunneled these leads, and exposed and fixed the distal ends on the back. For continuous rapid atrial pacing, we fastened a unipolar screw-in lead (CapSureFix 5568; Medtronic, Minneapolis, MN, USA) into the endocardial side of the RA appendage through the right external jugular

vein and connected it to a rapid pulse generator (Soletra, Medtronic). We performed all procedures in accordance with the guidelines specified by the Animal Experimentation and Ethics Committee of the Kitasato University School of Medicine [2, 6, 7].

Grouping of the objects

After a 1-week recovery period from the procedure, we initiated the atrial rapid pacing (400 beats/min) in the seven dogs. We divided the dogs into two groups: a pacing control group ($n=3$), in which the dogs received no additional medications, and a liraglutide group ($n=4$), in which the dogs received liraglutide (150 μg/kg/day) starting 3 days before the initiation of the rapid pacing. The liraglutide was subcutaneously injected once a day. We subcutaneously injected an equal volume of saline as that of the liraglutide dose and at the same frequency to the dogs in the pacing control group (Fig. 1).

Electrophysiological studies

We performed electrophysiological studies every week to evaluate AF inducibility, atrial effective refractory period (AERP), and conduction velocity (CV). We evaluated the incidence of AF induction with atrial burst pacing (delivered at fourfold the diastolic threshold with a pulse width of 2 ms) for 3 s at the minimal pacing cycle length that achieved a 1:1 atrial capture at the RA pacing site in order to evaluate AF inducibility. We measured the duration of detected AFs, and defined AF as a spontaneous irregular atrial rhythm lasting longer than 5 s. We delivered the atrial burst pacing for AF induction five times at the RA pacing site. At each evaluation time point, we measured the AERP with basic drive cycle lengths of 300 ms at the LA sites where the electrodes were sutured. We set the pacing energy output at twice the diastolic threshold during each evaluation for the RA pacing site (the coupling interval of the premature stimulus was shortened by 2 ms steps). We determined the longest coupling interval of the premature beat that failed to capture the atrium as the local AERP. We measured conduction times between the LA and RA during LA pacings at cycle lengths of 300, 200, and 150 ms, and calculated the CV as the reciprocal of conduction time between the two atrial recording sites (the values were expressed as the %CV after dividing each piece of data by the data for day 0 to exclude the influence of the difference in the actual distance of the electrodes in each dog).

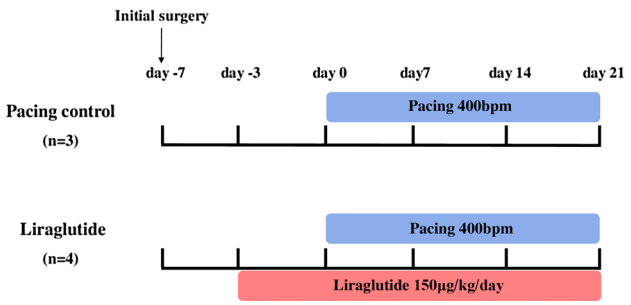

Fig. 1 Schematic of the study protocol. Each dog underwent initial surgery and was allowed to recover for 1 week without pacing before the start of atrial rapid pacing (day 0). Atrial rapid pacing (400 bpm) was performed for 3 weeks in the pacing control and liraglutide groups. In the liraglutide group, liraglutide (150 μg/kg/day) was administered by subcutaneous injection from day 3 until day 21

Hemodynamic evaluation

At the end of the whole study protocol, we evaluated hemodynamic variables including systemic blood pressure, pulmonary arterial pressure, pulmonary arterial wedge pressure, and cardiac output using a thermodilution catheter in all dogs.

Statistical analysis

We performed all statistical analyzes using the JMP statistical software (SAS Institute, Cary, NC, USA). We presented the values as means ± standard deviation or standard error. We used the nonparametric Wilcoxon test to analyze the basic comparative statistics for continuous data. We considered p values < 0.05 as statistically significant.

Results

Electrophysiological studies

Figure 2 shows the results of the electrophysiological studies. AF inducibility increased gradually in the pacing control group dogs, but it was suppressed in the dogs of the liraglutide group ($p=0.0262$). The pacing control group dogs displayed decreased CVs with time. In contrast, the dogs in the liraglutide group displayed almost unchanged CVs during the 3 study weeks. The average CVs in the liraglutide group became significantly higher than those in the pacing control group at 2 ($p=0.0323$) and 3 week ($p=0.0339$) time points. The pacing control group dogs exhibited gradual AERP shortenings with time, and the dogs in the liraglutide group experienced similar AERP shortenings; we found no significant differences between the two groups ($p=0.5926$).

Hemodynamic evaluation

Table 1 shows the hemodynamic variables in the pacing control and liraglutide groups evaluated at the end of the

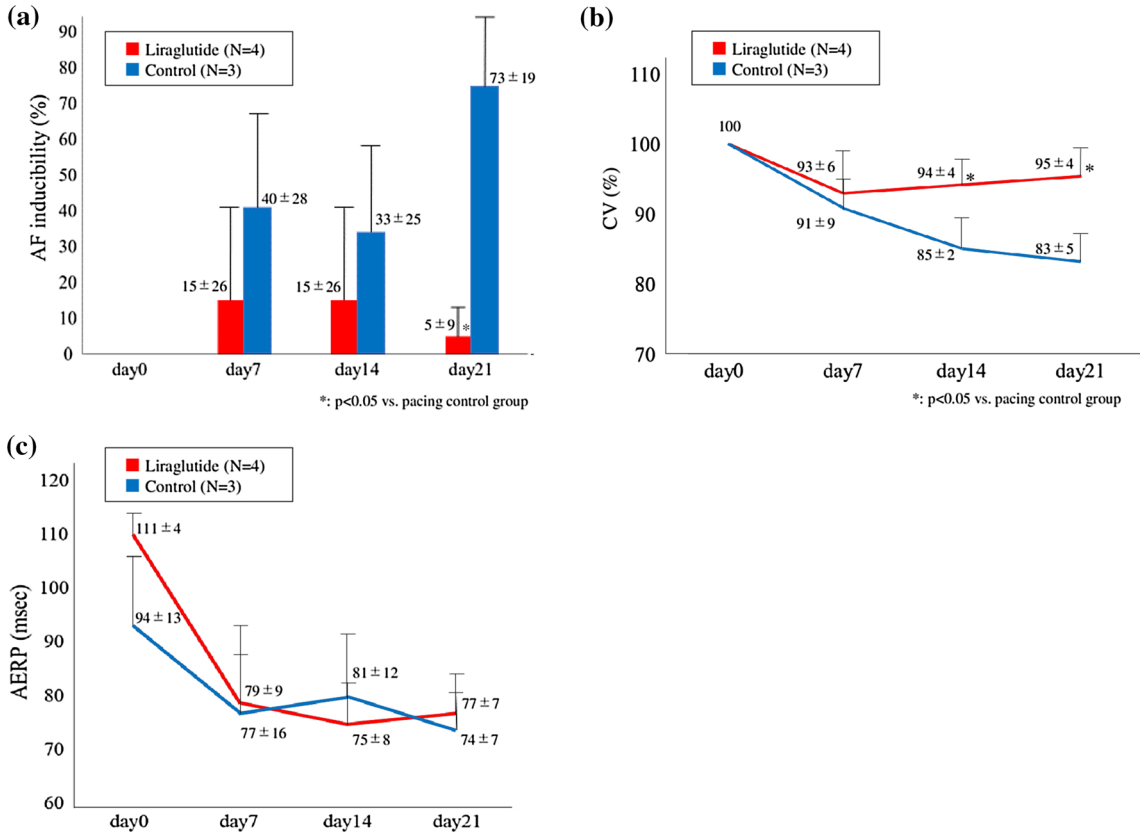

Fig. 2 Parameters in the electrophysiological studies. Increase in AF inducibility, CV decrease and gradual AERP shortening were observed in the pacing control group with time course of the rapid pacing. In contrast, in the liraglutide group, increase in AF inducibility and CV decrease were suppressed compared with the pacing control although the change in AERP did not exhibit significant difference. See text for details

Table 1 Hemodynamic parameters

	Liraglutide ($n=4$)	Pacing control ($n=3$)	p value
Systolic BP (mmHg)	136 ± 16	173 ± 18	0.1573
Systolic PAP (mmHg)	28 ± 6	30 ± 7	0.4795
Diastolic PAP (mmHg)	11 ± 4	18 ± 4	0.2888
PAWP (mmHg)	8 ± 3	9 ± 3	0.2253
CO (L/min)	4.1 ± 1	3.6 ± 1	0.4795

Data were given as mean ± SE

BP blood pressure, *CO* cardiac output, *PAP* pulmonary arterial pressure, *PAWP* pulmonary arterial wedge pressure

p value shows the difference among three groups

3-week pacing protocol. Although some dogs exhibited somewhat deviated data, we found no significant differences between the two groups in terms of these variables.

Discussion

Our study demonstrated two important findings: First, we successfully established the canine AF model using the 3-week rapid atrial pacing with electrical remodeling characterized by AERP shortening, CV decreases, and increases in AF inducibility as published [2, 6, 7]. Second, the liraglutide suppressed the AF inducibility and CV decreases in the model. Our findings suggest that the electrical remodeling of the atria may be modified by the administration of a GLP-1 analog.

AF has been associated with intracellular calcium (Ca^{2+}) homeostasis abnormalities. During the rapid atrial pacing, the high atrial rate causes accumulation of intracellular Ca^{2+} and down-regulation of L-type Ca^{2+} current (I_{CaL}) [8–10]. Reduced I_{CaL} decreases the inward Ca^{2+} current that should maintain the action potential (AP) plateau, shortens the AP duration, and thereby promotes an arrhythmogenic substrate of reentry. Reports have shown that atrial tachypacing decreases the sodium (Na^+) current (I_{Na}) and changes conduction velocities [8, 10]. Yue et al. demonstrated that these I_{Na} reductions occur concurrently with decreases in mRNA

expressions of factors encoding the Na+ channel α-subunit resulting in decreased protein levels [11]. Decreases in the I_{Na} may promote AF by decreasing the conduction velocity and promoting reentry through wavelength shortening [8, 12, 13]. In the present study, we reconfirmed these electrophysiological findings reflected in the AERP shortening, CV decreases, and AF inducibility increases in our canine AF model dogs. Additionally, the inflammatory process caused by hyper-oxidative stress is thought to promote interstitial proliferation and fibrosis resulting in atrial structural remodeling and formation of an arrhythmogenic substrate in our canine AF model. We also have reported the importance of such hyper-oxidative state in promoting the atrial remodeling [2, 6, 7].

We have reported the suppressive effect of linagliptin, a DPP4-inhibitor, on the progression of atrial remodeling in the same canine AF model [2]. We speculated that the pleiotropic effects of linagliptin might exhibit such anti-remodeling effects through its anti-oxidative effect. However, because the use of DPP4-inhibitor increases the intrinsic GLP-1 level, we thought GLP-1 itself might exhibit an anti-remodeling effect through direct action. GLP-1 has been shown to regulate arrhythmogenesis through modulation of Ca^{2+} handling in cardiac cells. Huang et al. demonstrated cardiac electrical effects for GLP-1 in HL-1 atrial myocytes using patch clamp analyzes [14]. GLP-1 administration increased the Ca^{2+} transient and sarcoplasmic reticular Ca^{2+} contents and decreased the phosphorylation of the ryanodine receptor. Consequently, the GLP-1 may suppress AF inducibility and CV decreases by modulating Ca^{2+} handling proteins. Moreover, reports have demonstrated the anti-oxidative effects of GLP-1 [15–18]. Fujita et al. reported that liraglutide decreased levels of superoxide and NAD(P)H oxidase and that it elevated cAMP and PKA activity in diabetic nephropathy mice model [15]. Our data seem to support the anti-oxidative stress function and suggest an effect of the DPP4-inhibitor in our model [2].

Limitations

We are aware of the limitations of our study. First, the influence of tachycardia-induced heart failure cannot be ruled out in our canine AF model because we did not perform His-bundle ablations. However, we did not find significant differences in hemodynamic variables, at least until the end of the study protocol, in this or in our previous studies [2, 6, 7]. We believe that this model mimics well the clinical AF in humans and it may be suitable for evaluating the possible clinical effects of drugs. Second, because the liraglutide dose in this study was single, dose response could not be determined. Additionally, we cannot know whether the clinical dose of liraglutide causes similar anti-remodeling effects in humans. Third, although we demonstrated the effects of liraglutide on the electrophysiological variables, we did not evaluate the effects on the oxidative stress and/or on interstitial fibrosis. Finally, we did not discuss the effects of liraglutide on the cellular electrical remodeling, because we did not examine the expressions of ionic channels. These pathological and molecular assessments should be evaluated in future studies with different settings using a patch-clamp technique for cellular electrophysiology, as well as Western blot analyzes and/or RT-PCR assays to obtain expression levels of variously related molecules such as SCN5A, L-type Ca, Kv 4.2, and others.

Conclusion

Liraglutide suppressed AF inducibility and CV decrease in the canine AF model. GLP-1 analogs may have protective effects on electrical remodeling of atria.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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