

## Mixed Endothelin A/B Receptor Antagonist Bosentan and Endothelin A Receptor Antagonist LU 135.252 Suppresses Intrapericardial Endothelin-1 Induced Ventricular Arrhythmias

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### Summary

*In earlier studies severe ventricular arrhythmias developed during intrapericardial (ip.) endothelin-1 (ET-1) infusion. Monophasic action potential duration (MAPD90) increase and significant ST segment elevation preceded the onset of arrhythmias. The aim of the present study was to test the antiarrhythmic and antiischemic efficacy of the mixed ET A/B-receptor antagonist Bosentan (BOS) and selective ET A receptor antagonist LU 135.252 (LU) on ET-1 induced arrhythmias. 10 minutes after an iv. bolus of either BOS (10 mg/kg, n = 6) or LU (5 mg/kg, n = 6) ET-1 (33 pmol/kg/min) was given into the pericardial space for 30 minutes (BOS and LU groups). Six control dogs received only ET-1 infusion (ET group). Arterial blood pressure (MABP), cardiac output (CO), ECG, right and left ventricular endo- and epicardial (RVendo, RVepi, LVendo, LVepi) MAPD90-values were recorded. MABP and CO did not change significantly in the BOS and LU groups. Significant MAPD90 prolongation was found in all investigated regions of the ET group (ET start vs. ET 20 min: LVepi:  $174 \pm 3$  vs.  $208 \pm 10^*$ , RVendo:  $206 \pm 9$  vs.  $241 \pm 12^*$  ms,  $*P < 0.05$ ), while significant MAPD90 alterations were not observed in BOS and LU groups (basic vs. ET 20. min in BOS group: LVepi:  $199 \pm 5$  vs.  $199 \pm 4$ , RVendo:  $194 \pm 5$  vs.  $195 \pm 6$ , and in LU group: LVepi:  $189 \pm 8$  vs.  $201 \pm 11$ , RVendo:  $191 \pm 10$  vs.  $192 \pm 9$ ). Early after-depolarizations were observed only in three dogs in ET group. Severe ventricular arrhythmias (incessant non-sustained ventricular tachycardias (nsVT) in all cases, sustained VTs in four, ventricular fibrillation in two instances) were present in the ET group, whereas nsVTs were observed only in two and one dogs in the BOS and LU groups, respectively. ST segment elevation was more pronounced in the control group than in the BOS and LU groups ( $10.1 \pm 2$  vs.  $4.1 \pm 0.65$  mV,  $P < 0.05$  and  $10.1 \pm 2$  vs.  $6.7 \pm 0.9$  mV). In summary, BOS and LU effectively inhibit ip. ET-1 induced ventricular arrhythmias, moreover they may have a protective effect against epimyocardial ischemia.*

### Key Words

Endothelin-1, ventricular arrhythmia, monophasic action potential, bosentan, LU 135.252

### Introduction

Endothelin-1 (ET-1), a 21-amino-acid peptide, was originally isolated from the supernatant of cultured porcine aortic endothelial cells. ET-1 has multiple actions on the heart. The peptide induces potent coronary vasoconstrictor and moderate inotropic and chronotropic effects. In anesthetized dogs, intracoronary ET-1 infusion induces severe ventricular arrhythmias [1,2,5,6,7,8,17]. Our workgroup has recently reported that canine and human pericardial fluid con-

tains high concentrations of ET-1 and intrapericardial infusion of ET-1 can induce severe ventricular tachyarrhythmias in dogs [1,7,10-13]. The pathomechanism of these arrhythmias seemed to be different from the direct (ischemic) arrhythmias, as well. In addition, accumulating body of evidence suggests that ET-1 may have direct arrhythmogenic effect [1,2,7,8,16].

Bosentan is a nonpeptide competitive antagonist, which is specific for the endothelin system and blocks

Time	MAPD <sub>90</sub> (ms)			
	Control	0 min	20 min	60 min (n = 4)
RV epicardial	172 ± 6	168 ± 7	204 ± 6**	176 ± 6
LV epicardial	176 ± 5	174 ± 3	208 ± 10**	182 ± 6
RV endo-cardial	199 ± 6	206 ± 9	241 ± 12**	204 ± 5
LV endo-cardial	203 ± 5	208 ± 5	254 ± 10**	211 ± 6

a

Time	MAPD <sub>90</sub> (ms)			
	Control	0 min	20 min	60 min
RV epicardial	189 ± 6	189 ± 5	196 ± 5	191 ± 5
LV epicardial	199 ± 7	199 ± 5	199 ± 4	197 ± 6
RV endo-cardial	194 ± 7	194 ± 5	195 ± 6	197 ± 4
LV endo-cardial	209 ± 6	209 ± 3	213 ± 5	210 ± 5

b

Time	MAPD <sub>90</sub> (ms)			
	Control	0 min	20 min	60 min
RV epicardial	187 ± 5	186 ± 7	190 ± 7	188 ± 5
LV epicardial	199 ± 7	189 ± 8	201 ± 11	195 ± 7
RV endo-cardial	192 ± 5	191 ± 10	192 ± 9	194 ± 4
LV endo-cardial	196 ± 8	199 ± 11	203 ± 11	205 ± 7

c

Table 1. Changes of electrophysiological parameters in groups A and B. a) ET group: 30 min intrapericardial ET-1 infusion (n = 6). b) BOS group: iv. bosentan and 30 min intrapericardial ET-1 infusion (n = 6). c) LU group: iv. LU 135.252 and 30 min intrapericardial ET-1 infusion (n = 6). \*\* P < 0.01.

the actions of endothelin at both mammalian receptors (A and B). In experimental models of heart failure bosentan acts as a vasodilator and neurohormonal blocker that improves overall left ventricular performance [4]. Furthermore, in chronic studies, bosentan

attenuates cardiac remodeling and significantly improves survival. Clinical trials in hypertensive patients indicate that bosentan reduces blood pressure without heart rate increase or neurohumoral stimulation [4]. LU 135.252 (LU) is a specific antagonist of ET A receptors. In earlier studies bolus injection of LU 135.252 decreased the probably endogenous ET mediated reperfusion arrhythmias following 90 min LAD occlusion [9].

Monophasic action potential (MAP) recording is suitable for studying the characteristics of local myocardial repolarization and identifying the bases for triggered ventricular arrhythmias, moreover this method is one of the most informative and sensitive in vivo electrophysiological method to detect possible myocardial ischemia [1,2,14,15].

The aim of the present study was to test the antiarrhythmic and antiischemic efficacy of the mixed ET A/B-receptor antagonist Bosentan and the selective ET A receptor antagonist LU 135.252.

## Materials and Methods

Acute experiments were conducted on three groups of sodium pentobarbital anesthetized and room air ventilated open chest mongrel dogs (weight: 21.6 ± 0.7 kg). Continuous recordings of blood pressure (BP), heart rate (HR), cardiac output (CO) and ECG were performed by PC.

Fractally coated monophasic action potential (MAP) catheters (YP-60-BP, Biotronik, Germany) were inserted through the right internal jugular vein into the apex of the right and left ventricles. After incising the pericardial sac fractally coated epicardial MAP electrodes (MAPOX, Biotronik) were placed onto the left and right anterolateral epicardium and a cannula was inserted into the pericardial space onto the anteroseptal area. The duration of MAP was determined during programmed electrical stimulation (PES) at 90 % repolarization (MAPD<sub>90</sub>).

Effects of 30 min intrapericardial ET-1 infusion (33 pmol/kg /min) was investigated in six dogs (ET group). After the beginning of ET-1 infusion, MAP and hemodynamic parameters were determined in 5 min intervals by PES.

In BOS and LU groups effects of the mixed ET A/B receptor antagonist Bosentan (BOS) and effects of the selective ET A receptor antagonist LU 135.252 (LU) were studied on ET-1 induced arrhythmias in mongrel dogs. 10 min after an iv. bolus of either BOS

(10 mg/kg, n = 6) or LU (5 mg/kg, n = 6), ET-1 (33 pmol/kg /min) was given into the pericardial space for 30 minutes. The 30 min ET-1 infusion was followed by a 60 min post infusion period in all groups when the determination of MAPD90 and hemodynamic parameters was again done in 5 minute intervals.

All results are expressed as mean  $\pm$  standard error of the mean. Differences between means in two groups at chosen time points were evaluated using analysis of variance for repeated measures.  $P < 0.05$  was considered to be statistically significant.

## Results

ET group: Intrapericardial ET-1 infusion caused severe ventricular arrhythmias in the ET group. Ventricular extrasystoles (VES) and incessant non-sustained ventricular tachycardias (nsVT) were observed in all cases, moreover sustained VT in four, ventricular fibrillation (VF) in two instances were noticed. VT had two or more different morphology suggesting multifocal activity in four cases. Mono- and polymorphic arrhythmias often alternated. CO and mean arterial blood pressure (MABP) did not change significantly at the onset of arrhythmias, later on when more severe arrhythmias occurred MABP and CO tended to decreased but failed to reach significance (MABP:  $132 \pm 3$  vs.  $130 \pm 4$  mmHg and CO:  $1.96 \pm 0.22$  vs.  $1.73 \pm 0.22$  l/min – control vs 20 min values,  $P = ns$ ). Significant MAPD90 prolongation was found in all investigated regions (Table 1). Early afterdepolarizations (EAD) were noticed in three dogs. ST segment elevation was pronounced in this group ( $10.1 \pm 2$  mV).

BOS and LU groups: Intrapericardial ET-1 infusion did not cause severe ventricular arrhythmias in BOS and LU groups. VES were observed in three and two nsVT were noticed in two and one dogs in BOS and LU groups, respectively. Sustained VT or VF were not present in BOS and LU groups. CO and mean arterial blood pressure (MABP) did not change significantly throughout the whole study (MABP:  $138 \pm 4$  vs.  $132 \pm 3$  mmHg and  $151 \pm 10$  vs.  $148 \pm 9$  mmHg, and CO:  $2.05 \pm 0.25$  vs.  $2.12 \pm 0.28$  l/min and  $2.18 \pm 0.36$  vs.  $2.10 \pm 0.34$  – control vs 20 min values in BOS and LU groups,  $P = ns$ ). MAPD90 did not lengthen in BOS and LU groups, EADs were not observed (Table 1). ST segment elevation was significantly higher in ET group than in either BOS or LU groups, and it was significantly lower in BOS group than in LU group (ET vs.

BOS group:  $10.1 \pm 2$  vs.  $4.1 \pm 0.65$  mV,  $P < 0.05$ , ET vs. LU group:  $10.1 \pm 2$  vs.  $6.7 \pm 0.9$  mV,  $P < 0.05$ , BOS vs. LU group:  $4.1 \pm 0.65$  vs.  $6.7 \pm 0.9$  mV,  $P < 0.05$ ).

## Discussion

Arrhythmogenic effect of intrapericardial ET-1- applied either in low (11 pmol/kg per minute) or high (33 pmol/kg per minute) dose – was proved in recent studies [1,7]. This effect was based on monophasic action potential and QT lengthening and early afterdepolarization formation. When applying low dose intrapericardial ET-1 neither ischemic ECG nor hemodynamic changes were observed at the onset of arrhythmias.

In earlier studies endothelin receptor antagonists have been proposed for the treatment of a variety of cardiovascular disorders. Endothelins may act as pathogenic mediators in different conditions such as congestive heart failure, systemic and pulmonary hypertension, and cerebral vasospasm, however bosentan was not able to prevent ischemia-reperfusion initiated arrhythmias and did not decreased infarct size in a rat model [3,4]. However, LU decreased the incidence of reperfusion arrhythmias following 90 min LAD occlusion in an experimental model [9].

Antiarrhythmic effect of both the mixed ET A/B antagonist bosentan and the selective ET-A antagonist LU 135.252 was proved in the present study, BOS and LU eliminated severe ventricular arrhythmias, while VES-es and nsVT did not disappear. MAPD90 values did not change significantly, afterdepolarizations were not present in the BOS and LU pretreated groups. The significant ST elevation in BOS and LU groups are presumably due to a direct local epimyocardial excitement on the site of the ET-1 infusion [16].

Findings of the present study further support the postulated direct arrhythmogenic pathomechanism of intrapericardial ET-1, and on the other hand these results further emphasize the possible role of ET-1 in arrhythmogenesis, and the importance of mixed A/B and selective A endothelin receptor antagonists in preventing severe ventricular tachyarrhythmias.

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