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Short communication

Endothelin-1 and unstable angina: Effect of either endothelin ET_A or ET_B receptor antagonism in a locally injured canine coronary artery

Michael Kirchengast [a,*], Stefan Hergenröder [a], Sabine Schult [a], Klaus Münter [a], Klaus Rübsamen [b]

[a] *Preclinical Cardiology, Knoll AG, P.O. Box 210805, D-67008 Ludwigshafen, Germany*
[b] *Clinical Angiology, Research and Development, Knoll AG, Ludwigshafen, Germany*

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Abstract

The role of endogenous endothelin-1 in variant angina was investigated using two endothelin receptor antagonists: LU 135252 (ET_A) and BQ 788 (ET_B). Cyclic flow reductions were induced in a coronary artery of mongrel dogs by combining critical stenosis with endothelial injury. One hour after induction of cyclic coronary flow reductions the dogs were randomized to intravenous treatment with either saline, or LU 135252 (10 mg kg^{-1}), or BQ 788 (0.1 mg kg^{-1}). Cyclic coronary flow reductions were monitored for two hours after drug and remained constant in controls as well as after BQ 788. LU 135252 reduced the number of cyclic coronary flow reductions significantly (about 50%) without effects on hemodynamics or hemostasis. © 1998 Elsevier Science B.V.

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1. Introduction

Adhesion and aggregation of platelets at sites of intimal injury is accompanied by local generation of vasoconstrictory substances like serotonin, thrombin, or endothelin-1 and thus vasospasm. Endothelin-1, well known since its discovery (Yanagisawa et al., 1988) to be the most potent vasoconstrictive endogenous peptide, could thus play a role in the genesis and maintenance of unstable angina. The effects of endothelin-1 are mediated via two different receptors, ET_A and ET_B. The endothelin ET_A receptor is located predominantly on vascular smooth muscle cells, coupled to G proteins, and promoting vasoconstriction as well as hypertrophy and hyperplasia (Rubanyi and Polokoff, 1994). The endothelin ET_B receptor, in the cardiovascular system primarily to be found on endothelial cells, is linked to the formation of vasodilator substances such as nitric oxide and prostacyclin (Rubanyi and Polokoff, 1994). The aim of the present study was to evaluate the effect of the ET_A receptor antagonist LU 135252 (Münter et al., 1996), and the ET_B receptor antagonist BQ 788 (Ishikawa et al., 1994) on intraarterial heart thrombus formation and vasoconstriction in critically stenosed coronary arteries with locally damaged endothelium in anaesthetized mongrel dogs. This model was chosen as it represents an accepted method to experimentally mimic the clinical situation of unstable angina (Folts, 1991).

2. Methods

The experimental protocol of this study was approved by the local ethics committee (Tierschutzkomission der Bezirksregierung Rheinhessen–Pfalz) and conformed to the guidelines for the care and use of laboratory animals published by the US National Institutes of Health (Institute of Laboratory and Animal Resources, Commission on Life Science, National Research Council (USDHHS, PHS, NIH Publication No 85-23, revised 1985).

2.1. Surgical preparation

The method used in these experiments has been first described by Folts et al. (1976) and was slightly modified to suit our requirements (Kirchengast et al., 1994). Briefly,

* Corresponding author. Tel.: +49-621-5892879; Fax: +49-621-5893551; e-mail: michael.kirchengast@knoll-ag.de.

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16 mongrel dogs weighing 11.5–15.0 kg were screened 3–5 d before the individual experiment to ensure that only animals with platelet counts greater than 2×10^5 μl^{-1} were enrolled. The animals were anaesthetized by an intravenous injection of 30 mg kg^{-1} pentobarbital sodium (Nembutal®, Sanofi). Anaesthesia was maintained by continuous infusion of 3 mg kg^{-1} h^{-1} pentobarbital. After intubation, the animals were ventilated with a Bird respirator (Mark 8) using a mixture of 25% O_2 and 75% N_2O. Body temperature was maintained at 38.5 ± 0.5°C with a temperature-controlled heating table. After thoracotomy in the fifth left intercostal space a pericardial cradle was formed and the left anterior descending coronary artery was dissected from the myocardium at a length of approximately 2 cm. An electromagnetic flow probe (Narcomatic™) was positioned at the proximal portion of the artery to allow continuous recording of mean left anterior descending coronary artery blood flow. Thereafter, a segment of this artery distal to the flow probe was traumatized using armed forceps. Cyclic coronary blood flow reductions immediately developed when a plexiglass cylinder of suitable size, chosen such as to abolish the hyperemic response after a short complete occlusion, was placed around the left anterior descending coronary artery at the site of endothelial injury.

2.2. Experimental protocol

After induction of stable cyclic coronary flow reductions baseline values for heart rate, systolic and diastolic arterial blood pressure, left ventricular enddiastolic pressure, left ventricular contractility, mean left anterior descending coronary artery blood flow, and cyclic coronary flow reductions were continuously measured during one hour. The animals were then randomly allocated to one of the three treatment groups and received either isotonic NaCl solution (control; $n = 6$), or LU 135252 (10 mg kg^{-1}; $n = 6$), or BQ 788 (0.1 mg kg^{-1}; $n = 4$) as intravenous injection over 2 min into the brachial vein. All parameters were recorded over another two hours.

2.3. Dose finding

The dose of BQ 788 was determined in an experimental series in pitched rats. The peptidic endothelin ET_B receptor selective agonist sarafotoxin S6c (1.0 μg kg^{-1}) was injected intravenously either without or with pretreatment with different doses of BQ 788 (0.01–1.0 mg kg^{-1}). From this dose response in rats it was seen that 0.1 mg kg^{-1} BQ 788 was able to block the blood pressure response to sarafotoxin S6c by more then 75%. This dose was then tested in two dogs at the end of another experimental series and found to almost completely block endothelin ET_B receptor mediated responses. It was thus used in the experiments reported in this paper.

2.4. Platelet aggregation

Platelet aggregation in platelet rich plasma in response to several agonists (adenosine diphosphate, thrombin, and the stable thromboxane analogue U 46619; for methodological details see (Kirchengast et al., 1994)) was measured turbidimetrically at 37°C in a dual chamber aggregometer before induction of cyclic coronary flow reductions, before drug and 10 and 60 min after drug administration.

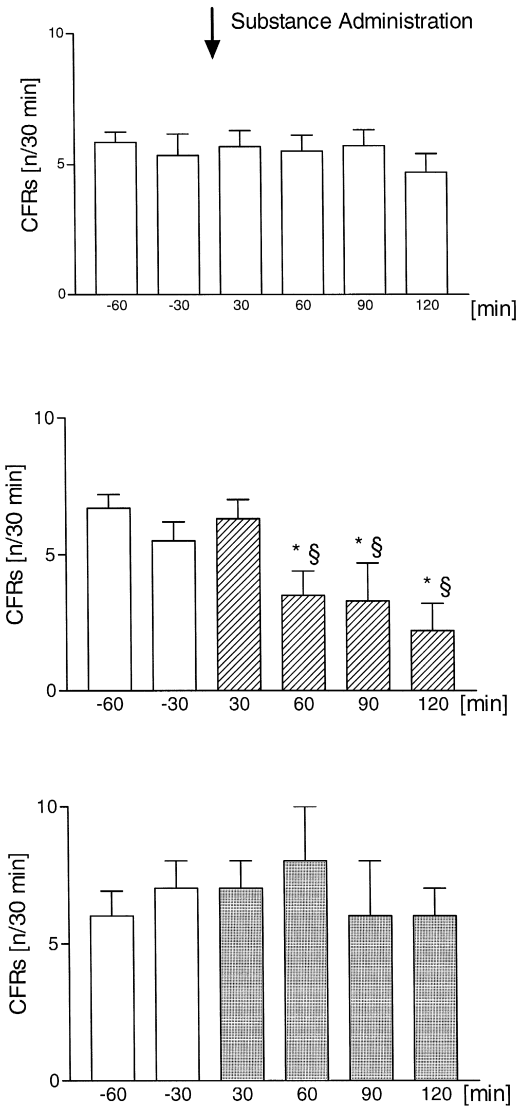

Fig. 1. Cyclic coronary flow reductions (CFRs) in dogs treated intravenously with either isotonic saline solution (control; $n = 6$; open bars), or the endothelin ET_A receptor antagonist LU 135252 (10 mg kg^{-1}; $n = 6$; hatched bars), or the endothelin ET_B receptor antagonist BQ 788 (0.1 mg kg^{-1}; $n = 4$; dotted bars). Values are given as absolute numbers of cyclic coronary flow reductions per 30 min for the 1 h pretreatment and the 2 h posttreatment periods. Mean ± S.E.M. $^{*}P < 0.05$ versus pretreatment period; $^{\S}P < 0.05$ versus the other treatment groups at the same period of time.

Table 1
Hemodynamic parameters before and 60 min after intravenous application of either 0.9% NaCl (Control), 10 mg kg^{-1} LU 135252 and 0.1 mg kg^{-1} BQ 788

Parameter	Control ($n = 6$)	Control 60 min ($n = 6$)	LU 135252 predrug ($n = 6$)	LU 135252 60 min ($n = 6$)	BQ 788 predrug ($n = 4$)	BQ 788 60 min ($n = 4$)
HR (min^{-1})	130 ± 11	131 ± 7.1	139 ± 8.2	134 ± 7.6	118 ± 10	112 ± 10
SAP (mm Hg)	112 ± 8.4	117 ± 8.7	102 ± 9.1	98 ± 7.8	117 ± 4.0	114 ± 4.5
DAP (mm Hg)	84 ± 6.6	90 ± 6.1	72 ± 5.6	71 ± 5.7	86 ± 7.8	89 ± 12
LVEDP (mm Hg)	5.3 ± 0.6	5.7 ± 0.6	4.2 ± 1.2	3.2 ± 1.7	3.5 ± 1.2	2.8 ± 0.9
LV$dP/dt_{max}$ (mm Hg/s)	2830 ± 305	2830 ± 345	2560 ± 442	2840 ± 748	2230 ± 103	2280 ± 131

HR = heart rate; SAP = systolic, and DAP = diastolic arterial blood pressure; LVEDP = left ventricular enddiastolic pressure; LV$dP/dt_{max}$ = left ventricular contractility.
Data are mean ± S.E.M.

2.5. Drugs

LU 135252 ((+)-(*S*)-2-(4,6-dimethoxy-pyrimidin-2-yloxy)-3-methoxy-3,3-diphenyl-propionic acid) was synthesized by BASF, Ludwigshafen; BQ 788 (*N-cis*-2,6-dimethylpiperidinocarbonyl-L-γMeLeu-D-Trp(COOMe)-D-Nle-ONa)) and sarafotoxin S6c were purchased from Alexis (Switzerland). LU 135252 was dissolved in aqueous solution containing 0.1 N NaOH and later buffered by addition of HCl to a pH between 7.2 and 7.4. The other two compounds were dissolved in distilled water.

2.6. Statistical analysis

Results are expressed as mean ± S.E.M. Comparisons between groups were made using between-within analysis of variance (ANOVA) and differences between individual groups were assessed by Newman–Keuls-test for multiple comparisons. Paired *t*-test was applied to the pre and post values of cyclic coronary flow reductions, and slope values of platelet aggregation. An error probability of < 0.05 was considered as statistically significant.

3. Results

The frequency of cyclic coronary flow reductions was not statistically different during the 60 min control periods before drug administration in the groups investigated and did not change over the two hour observation period following substance application in the control group (Fig. 1). Likewise, administration of BQ 788 had no effect on cyclic coronary flow reductions at all (Fig. 1). On the contrary, LU 135252 was able to reduce number and size of the cyclic coronary flow reductions reaching a maximum of about 50% reduction in the second hour after application. Endothelin ET_A receptor blockade had a slow onset of action; during the first 30 min after injection the effect developed slowly and reached a plateau at about 45 min post drug, as can be seen from Fig. 1.

None of the treatments had any detectable effect on pressure parameters or heart rate at any time after administration. In Table 1 the values at the end of the 60 min control period as well as at the end of the first hour after drug are given, to correspond with the periods in time shown for cyclic coronary flow reductions in Fig. 1. From these data a systemic hemodynamic cause for the differences between the two different endothelin receptor antagonists can be excluded. As, due to the fixed diameter of the left anterior descending coronary artery, the 3 different groups did also not significantly differ in blood flow through this artery between cyclic flow reductions (mean blood flow over all groups 25 ± 2 ml/min), a direct flow related effect can also be excluded.

Platelet aggregation was measured ex vivo in platelet rich plasma. There was no difference in the slope values using platelet rich plasma collected at time 0, before vessel injury and stenosis, and at the end of the 60 min control period with cyclic coronary flow reductions. Also neither endothelin ET_A receptor antagonism by LU 135252 nor endothelin ET_B receptor antagonism by BQ 788 had any influence on platelet aggregation 10 and 60 min after administration (data not shown).

4. Discussion

The aim of this study was to evaluate the efficacy of either endothelin ET_A or ET_B receptor antagonism on the occurrence of cyclic coronary flow reductions in anaesthetized dogs and thus to clarify if endogenous endothelin-1 had any role in this model of unstable angina. It was shown that blockade of endothelin ET_A receptors by LU 135252 was able to reduce cyclic coronary flow reductions by about 50%, whereas blockade of endothelin ET_B receptors by BQ 788 was totally ineffective.

The model used in this study has been well characterized pharmacologically (Folts, 1991; Kirchengast, 1994) and generally is accepted as a model of intracoronary thrombus formation and vasoconstriction. Employing essentially the same model it has been shown that exogenous, intravenously administered endothelin-1 (from 0.1 to 1.0 μg kg^{-1} i.v.) was dose dependently inhibiting the formation of intracoronary thrombi in anaesthetized dogs

(Leadley et al., 1995). Higher exogenous endothelin-1 doses (4 μg kg^{-1} min^{-1}) clearly led to coronary vasoconstrictions via stimulating the endothelin ET_A receptor in anaesthetized dogs with intact endothelium and without coronary stenosis (Cannan et al., 1996). These two findings are not in disagreement with our results, if one bears in mind that endothelin-1, when administered i.v., has easy access to endothelin ET_B receptors and by stimulating them will lead to an increase in NO- as well as prostacyclin-production from the endothelium, both being antithrombotic as well as vasodilatatory (Rubanyi and Polokoff, 1994). When administered in higher concentrations, however, endothelin ET_A receptor mediated vasoconstriction will override the dilatatory effects of endothelin-1 at the ET_B receptor, a finding already observed by Yanagisawa, who at that time, however, had no explanation for the dual action of exogenous endothelin-1 (Yanagisawa et al., 1988). In the present study the vasoconstriction induced by abluminally secreted endogenous endothelin binding to smooth muscle cell endothelin ET_A receptors was, at least partially, antagonized by blocking these receptors with LU 135252. Thus the local vasodilatatory effects of endothelin-1 circulating in the blood and still being able to bind to endothelin ET_B receptors might have contributed to the effect of LU 135252 on cyclic coronary flow reductions.

The finding that blockade of endothelin ET_A receptors had no influence on agonist induced ex vivo platelet aggregation is in accordance with the fact that up to now no definitive evidence for the existence of receptors for endothelin-1 on platelets has been published. One may speculate that the effect observed is mediated in vivo by an increase in NO and prostacyclin via endothelin ET_B receptor stimulation as discussed above. However, as both these substances exhibit a very short biological half-life an effect of either NO or prostacyclin can not be detected in ex vivo platelet aggregation tests. Separate experiments to investigate the effect of endothelin-1 and its receptors on coagulation parameters need to be done to clarify the role of this peptide in hemostaseology.

Tissue endothelin-1 was found to be significantly elevated within the atherosclerotic plaque in patients and it was hypothesized that this locally increased endothelin-1 was responsible for increased vasoconstriction in unstable angina (Zeiher et al., 1995). Therefore the results reported here may have clinical implications for patients with coronary artery stenosis with the risk of developing unstable angina. Clinical studies need to be conducted to show if application of an endothelin ET_A receptor antagonist like LU 135252 would be able to normalize increased vascular reactivity as well as to reduce the risk of developing acute intracoronary thrombosis in patients with unstable angina.

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