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Effect of long-term oral pretreatment with levosimendan on cardiac arrhythmias during coronary artery occlusion in conscious rats

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Abstract

Heart failure is frequently associated with cardiac arrhythmias. The aim of the present study was to investigate the effect of levosimendan, a new cardiotonic drug for the treatment of congestive heart failure, on experimental ischaemic arrhythmias. Acute coronary artery occlusion was produced in conscious rats 7–10 days after placement of ligature around the left main coronary artery. Acute pretreatment with levosimendan (0.2 or 0.6 mg/kg orally 1 h before coronary artery occlusion) did not influence the incidence, onset and duration of arrhythmias. Long-term pretreatment with levosimendan (0.2 or 0.6 mg/kg orally twice a day for 2 weeks) increased the survival rate (50% and 81% vs. 44% in controls) and the number of animals without any arrhythmia (37% and 31% vs. 5% in controls). The present results demonstrate that chronic oral treatment with levosimendan could be beneficial in congestive heart failure and arrhythmias resulting from regional myocardial ischaemia.
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Keywords: Myocardial infarction; Arrhythmias; Survival rate; (Rat); Levosimendan

1. Introduction

Long-term use of positive inotropic agents in patients with congestive heart failure resulted in conflicting outcome. Administration of β-adrenergic agonists (e.g. dobutamine, xamoterol) or phosphodiesterase III inhibitors (e.g. milrinone, enoximone) although improved the haemodynamics in these patients, failed to extend long-term survival, moreover resulted in increased mortality (David and Zaks, 1986; The Xamoterol in Severe Heart Failure Study Group, 1990; Packer et al., 1991).

Conventional positive inotropic agents (e.g. β-adrenergic agonists, phosphodiesterase inhibitors) increase myocardial contractility by enhancing cyclic adenosine monophosphate (cAMP) level and the free intracellular calcium concentration. They also increase the spontaneous release of Ca^{2+} from the sarcoplasmatic reticulum. These effects, together with the upregulated Na^{+}–Ca^{2+}-exchanger and the downregulated inward rectifying K^{+}-current (I_{K1}) may contribute to the development of delayed afterdepolarizations that may initiate fatal ventricular arrhythmias during heart failure (Pogwizd et al., 2001).

Dobutamine has been found to increase the incidence of ventricular tachycardia and fibrillation during myocardial ischaemia–reperfusion in isolated guinea-pig hearts (Du Toit et al., 1999) and in anaesthetised dogs after myocardial infarction and programmed electrical stimulation (Stump et al., 2000). Phosphodiesterase III inhibitors (e.g. amrinone, milrinone, pimobendane) also increased the incidence of ventricular arrhythmias occurring in dogs with recent myocardial infarction (Lynch et al., 1989; Trolese-Mongheal et al., 1992).

Levosimendan is a novel calcium-sensitizing cardiotonic drug, recommended currently for the short-term intravenous treatment of congestive heart failure (Follath et al., 2002), but its application for long-term oral administration is also under development (Lehtonen, 2000). In low concentration, levosimendan does not increase cytosolic free calcium level, but improves myocardial contractility by enhancing the

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sensitivity of the contractile apparatus to calcium (Haikala et al., 1995a,b; Hasenfuss et al., 1998; Levijoki et al., 2000). In higher concentrations, it also inhibits phosphodiesterase III (Edes et al., 1995) and opens ATP-dependent potassium channels (Yokoshiki et al., 1997a,b). The latter can cause peripheral vasodilatation and ATP sparing during myocardial ischaemia, therefore levosimendan may be regarded as a cardioprotective inotropic drug.

Oral administration of levosimendan to patients with severe congestive heart failure considerably improved haemodynamic parameters without increasing heart rate (Harjola et al., 1999). Clinical electrophysiologic investigations showed that the drug has no significant potential to provoke life-threatening arrhythmias during its intravenous use in heart failure patients (Singh et al., 1999). In isolated perfused guinea pig hearts levosimendan improved mechanical function during reperfusion after a short period of ischaemia, without increasing the incidence of arrhythmias (Du Toit et al., 1999).

The aim of the present experiments was to investigate the effects of a single dose and a 2-week oral administration of levosimendan on the occurrence of arrhythmias and survival in conscious rats with acute myocardial infarction.

2. Materials and methods

2.1. Animals

Male, Sprague–Dawley CFY rats, weighing 340–380 g were used. Animals were fed commercial laboratory rat food pellet and allowed to drink tap water ad libitum. The animals were handled according to a protocol reviewed and approved by the Ethical Committee for the Protection of Animals in Research of the Albert Szent-Györgyi Medical Center, University of Szeged, Hungary.

2.2. Coronary artery ligation

Our surgical technique (Leprán et al., 1983) is a modification of that described by Selye et al. (1960). During ether anaesthesia, the thorax was opened in the fourth intercostal space, and the heart was exposed by applying gentle pressure upon the thorax. A loose loop of atraumatic silk (Ethicon, K 890 H, 5-0, UK) was placed around the left main coronary artery, about 2 mm from its origin. The silk was previously led through the wall of a cylinder-shaped polyethylene tube and, after understitching the coronary artery, it was carried outside the thoracic cavity, with the tube remaining in the thorax. The heart was set back in place and the thorax was closed, while the chest was slightly compressed to stop pneumothorax and to recover spontaneous respiration. The mortality of this preliminary surgery is negligible (1 out of 80 animals in the present experiments), because of the loose silk loop at this time.

After complete recovery from the preliminary surgery (7–10 days), the loose ligature was tightened to produce acute coronary artery occlusion and myocardial infarction in conscious, freely moving animals.

A bipolar electrocardiogram was recorded continuously using subcutaneous electrodes on both sides of the chest wall. The survival rate and the incidence of arrhythmias were registered and evaluated in accordance with the Lambeth Conventions (Walker et al., 1988), i.e. as ventricular fibrillation (VF), ventricular tachycardia (VT) and other types of arrhythmias (VES), including ventricular premature beats, bigeminia, and salvos. The duration of VF, VT and other types of arrhythmias was also measured. An arrhythmia score was used to include the incidence and the duration of arrhythmias by giving a grade to the animals as follows: 0 = no arrhythmia; 1 = < 10 s VES and/or VT; 2 = 11–30 s VES and/or VT; 3 = 31–90 s VES and/or VT; 4 = 91–180 s

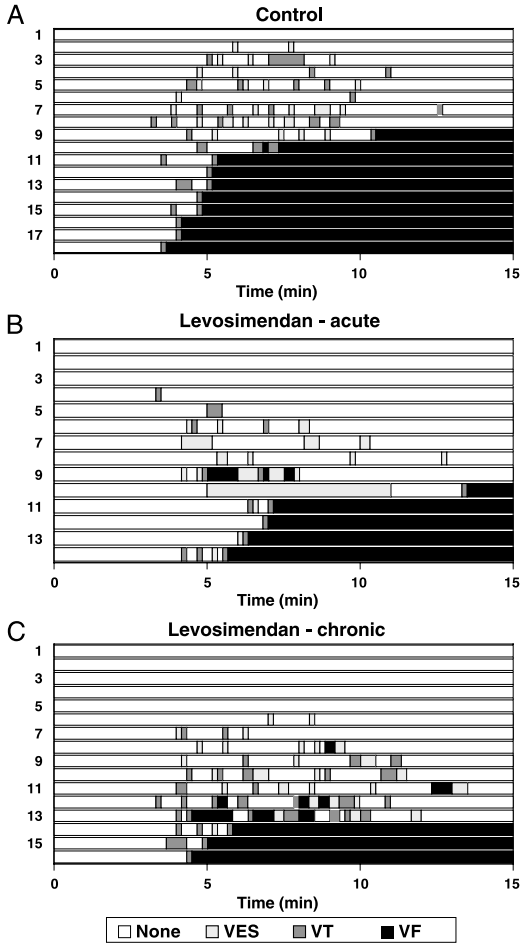

Fig. 1. The arrhythmia map of the control group (A) and groups of animals pretreated with a single dose (B) or twice a day for 2-weeks (C) with oral levosimendan (0.6 mg/kg). Each number of the ordinates refers to a separate animal and every row shows the arrhythmias of the given animal during the first 15 min after coronary occlusion in conscious rat. In each group the animals are listed in the order of severity of their arrhythmias. None = period without arrhythmia; VES = ventricular premature beats, salvos, and/or bigeminy; VT = monomorphic ventricular tachycardia; VF = ventricular fibrillation.

Table 1
Effect of acute or chronic oral administration of levosimendan on the survival rate and the incidence of arrhythmias during the acute phase of regional myocardial ischaemia in conscious rats

Group	Dose (mg/kg)	*n*	Survived (*N*/%)	Incidence of arrhythmias (*N*/%)				Arrhythmia score
				None	VF	VT	VES	
Control		18	8/44	1/5	11/61	16/89	8/44	4.44 ± 0.49
Levosimendan-acute	0.20	15	9/60	3/20	8/53	11/73	6/40	3.53 ± 0.67
	0.60	14	9/64	3/21	6/43	9/64	6/43	3.50 ± 0.64
Levosimendan-chronic	0.20	16	8/50	6/37[a]	8/50	10/62	7/44	3.50 ± 0.72
	0.60	16	13/81[a]	5/31[a]	7/44	9/56	9/56	3.00 ± 0.61

Levosimendan-acute: a single dose of the drug was applied orally 1 h prior to coronary artery ligation. Levosimendan-chronic: the drug was given orally twice a day for 14 days, the last treatment took place 1 h prior to coronary artery ligation.
n = total number of animals, *N* = number of animal exhibiting the given response. VF = ventricular fibrillation, VT = ventricular tachycardia, VES = other arrhythmias, including ventricular premature beats, salvos, and bigeminia together.
[a] Statistically significant difference ($P < 0.05$), calculated by the chi-square test.

VES and/or VT, < 10 s reversible VF; 5=>180 s VES and/or VT, >10 s reversible VF; 6 = irreversible VF.

No attempt was made to defibrillate the animals by taping on the chest wall in case of ventricular fibrillation.

2.3. Determination of the infarcted area

In the animals that survived for 16 h, the size of the infarcted area was measured using the method of Nachlas and Shnitka (1963). The rats were anaesthetised with pentobarbitone (60 mg/kg i.p.), and their hearts were excised and washed in isotonic saline solution. After cutting with razor to approximately 1-mm thin transversal slices, the pieces were stained in 0.1% nitroblue-tetrazolium dye. The wet weight of the unstained, i.e. infarcted myocardium was expressed as percent of the total weight of the ventricles.

2.4. Pretreatment

Levosimendan (Orion, Finland) was dissolved in Na_2HPO_4 solution (50 ml 2.32% $Na_2HPO_4 \times 2\ H_20$ with 300 μl 1 M NaOH) and was applied orally in a total volume of 5 ml/kg. During the chronic pretreatment the compound was given in doses of 0.2 or 0.6 mg/kg twice a day for 14 days, the last treatment occurred 1 h prior to the coronary artery ligation. In the course of the acute pretreatment the animals were given the Na_2HPO_4 solution as vehicle twice a day for 14 days, but the last treatment 1 h before the coronary artery occlusion also contained levosimendan (0.2 or 0.6 mg/kg). Control animals were treated with 5 ml/kg Na_2HPO_4 solution as vehicle twice a day for 14 days, the last administration took place 1 hour prior to the coronary ligation.

2.5. Statistical evaluation

The survival rate and the incidence of arrhythmias were compared by using the Chi-square method. Other parameters were expressed as mean ± standard error of the mean (S.E.M.), and after analysis of variance (one-way ANOVA), compared by means of the modified *t*-statistical test of Wallenstein et al. (1980) or by Gehan's generalized Wilcoxon test (Knapp and Wise, 1985). A value of $P < 0.05$ was considered significant.

3. Results

None of the conscious animals developed any arrhythmia before coronary artery ligation in the acute or chronic levosimendan or vehicle treated groups.

Coronary artery occlusion induced various arrhythmias, leading to ventricular fbrillation in 11 out of the 18 control animals (Fig. 1). These arrhythmias appeared around the

Table 2
Effect of acute and chronic oral administration of levosimendan on the appearance and length of arrhythmias in conscious rats surviving the acute phase of regional myocardial ischaemia

Group	Dose mg/kg	*n*	Appearance of arrhythmias (min)	Duration of arrhythmias (min)	Length of arrhythmic attacks (s)			
					VF	VT	VES	Total
Control		8	4.76 ± 0.63	5.15 ± 0.99	2.5 ± 2.5	39 ± 15.1	34 ± 10.0	75 ± 21.8
Levosimendan-acute	0.20	9	6.89 ± 1.11	2.70 ± 1.21	14 ± 9.6	38 ± 22.5	20 ± 7.5	72 ± 37.1
	0.60	9	7.07 ± 1.18	2.80 ± 1.04	10 ± 10.0	10 ± 4.1	34 ± 15.1	54 ± 23.0
Levosimendan-chronic	0.20	8	8.21 ± 1.36[a]	1.33 ± 0.91[a]	4.0 ± 3.7	37 ± 36.1	7.0 ± 6.2	49 ± 40.8
	0.60	13	7.65 ± 1.29	3.71 ± 1.04	22 ± 12.2	28 ± 10.8	38 ± 10.3	85 ± 30.5

n = number of animals survived. Mean values ± S.E.M.
[a] Statistically significant difference ($P < 0.05$), calculated by Student's *t*-test. For further details, see Table 1.

Table 3
Effect of acute and chronic oral administration of levosimendan on the alteration of heart rate (beats min^{-1}) in conscious rats surviving regional myocardial ischaemia

Group	Dose (mg/kg)	*n*	Basal	Ischaemia				
				1 min	3 min	5 min	10 min	15 min
Control		8	384 ± 11.6	390 ± 15.0	376 ± 16.6	395 ± 22.5	390 ± 15.0	374 ± 12.6
Levosimendan-acute	0.20	9	399 ± 11.2	410 ± 22.3	389 ± 13.9	370 ± 8.9	398 ± 11.1	393 ± 11.2
	0.60	9	437 ± 18.5[a]	424 ± 19.2	407 ± 20.0	464 ± 20.5	427 ± 20.1	412 ± 18.8
Levosimendan-chronic	0.20	8	389 ± 11.4	404 ± 13.2	378 ± 11.4	396 ± 10.4	388 ± 15.0	375 ± 13.9
	0.60	13	397 ± 9.7[b]	369 ± 14.9[b]	392 ± 13.8	391 ± 10.8	383 ± 7.8[b]	392 ± 8.1

n = number of animals survived. For further details, see Table 1.
[a] Statistically significant ($P < 0.05$) difference from the control group, calculated by Student's *t*-test.
[b] Statistically significant ($P < 0.05$) difference from the respective levosimendan-acute group, calculated by Student's *t*-test.

fourth to sixth minutes and terminated at the ninth to 11th minutes, if the animal survived the acute phase of myocardial infarction.

Acute oral pretreatment with levosimendan offered a mild protection against arrhythmias following coronary occlusion. The survival rate and the number of animals without developing any arrhythmia was slightly but not significantly increased (Fig. 1). The incidence of different arrhythmias did not change significantly, although the occurrence of ventricular fibrillation and tachycardia tended to decrease (Table 1). The onset of arrhythmias was also delayed to some extent (Table 2). This acute oral pretreatment with levosimendan increased the resting heart rate significantly and in a dose-related manner. The heart rate remained higher even during the initial period of myocardial infarction (Table 3).

Long-term oral administration of levosimendan offered more significant protection against the development of arrhythmias than the acute single dose treatment. The survival rate was significantly increased by the larger dose (2 × 0.60 mg/kg daily for 2 weeks), and the number of animals surviving without developing any arrhythmia was significantly enhanced by both applied doses (Table 1). The chronic pretreatment significantly delayed the appearance of the first arrhythmia and considerably shortened the duration of the period of dysrhythmias (Table 2). The tachycardiac response to levosimendan observed after a single dose treatment disappeared when the drug was administered chronically. The heart rate measured at the end of the two-week pretreatment with levosimendan did not differ significantly from the control values either before or after coronary occlusion (Table 3).

In the present experiments levosimendan pretreatment, applied either as a single dose or twice daily for 2 weeks, did not influence significantly the late mortality after coronary artery ligation. 6, 7, 6, 7 and 8 animals survived for 16 hours after coronary artery occlusion in the control, levosimendan-acute 0.20 and 0.60 mg/kg and levosimendan-chronic 0.20 and 0.60 mg/kg groups, respectively. The infarct size, measured at this time, did not vary significantly among the five different groups.

4. Discussion

The present results show that 2-week oral pretreatment with levosimendan exerted a significant protective effect against the development of arrhythmias resulting from acute myocardial infarction in conscious rats.

In contrast, our earlier experiments demonstrated that acute pretreatment with pimobendane, another positive inotropic agent, increased the risk of developing ventricular arrhythmias in the same model (Leprán and Papp, 1992). In general, our present results are inconsistent with the previous conclusion that, regardless of their mechanism of action, positive inotropic agents increase the risk of developing arrhythmias (Stump et al., 2000). The reason for this discrepancy may be the difference in the site and mode of action of the compounds investigated. The major mechanism of the positive inotropic effect of both β-adrenergic agonists and phosphodiesterase inhibitors is the increase of cAMP formation and consequently the free intracellular calcium concentration. This results in positive inotropic effect and tachycardia, thereby increasing the oxygen consumption of the heart. Levosimendan, however, does not influence the cAMP level or free calcium concentration intracellularly, at least in smaller therapeutic concentrations (Lancaster and Cook, 1997). Its main effect is to enhance calcium sensitivity of contractile proteins in cardiac muscle by calcium dependent binding of the drug to cardiac troponin C (Pollesello et al., 1994; Haikala et al., 1995a; Levijoki et al., 2000). This results in an increase in myocardial contractility, which due to the calcium dependency is not associated with impairment of relaxation. Further, as opposed to the marked tachycardiac effects of pimobendan and milrinone after coronary occlusion in conscious rats (Leprán and Papp, 1992), levosimendan causes only a small rhythmic increase in heart rate under the same experimental conditions which disappears upon chronic administration of the drug as seen in our present study. Levosimendan increases stroke volume and cardiac output without a significant increase in myocardial oxygen consumption (Ukkonen et al., 2000), and it may thus preserve the global contractile performance of the heart during acute myocardial ischaemia without an excessive increase in cardiac oxygen

consumption. Such an advantageous haemodynamic support may largely contribute to the anti-ischaemic–antiarrhythmic effect of the compound observed in the present experiments.

Levosimendan is able to produce vasodilatation in coronary arteries, thereby increasing collateral circulation to preserve myocardium after coronary artery ligation. However, collateral circulation in the rat heart is negligible (Maxwell et al., 1987). Moreover, we did not find a decrease in myocardial infarct size in the levosimendan treated and surviving animals. Therefore it is unlikely that opening collaterals to improve perfusion of the ischaemic myocardium would play a role in the antiarrhythmic effect of levosimendan in our experiments.

The present results demonstrate that the protective effect of levosimendan is particularly obvious upon a long-term treatment with the drug. It has become known that levosimendan produces an active metabolite (OR-1896) that may contribute to the positive inotropic effect of the parent drug in vivo. This metabolite is able to produce similar increase in myofibrillar calcium sensitivity as levosimendan (Takahashi et al., 2000) and presumably it also exhibits beneficial haemodynamic effects in ischaemic arrhythmias. Following the long-term oral pretreatment with levosimendan, we observed tolerance to the tachycardiac effect of the drug. Whether this effect is due to the active metabolite of levosimendan or to some desensitization of the phosphodiesterase enzyme needs further experimentation. The less tachycardia, however, can be advantageous during myocardial ischaemia and in congestive heart failure.

In conclusion, in conscious rats arrhythmogenic effect was not detected after oral administration of levosimendan, neither following acute nor after chronic pretreatment, in basal condition or after coronary occlusion. Long-term treatment with levosimendan proved to be protective against the development of regional myocardial ischaemia induced arrhythmias and improved the rate of survival from acute myocardial infarction.

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